

Literature Review

Chapter 2

1. Introduction: *Schizophrenia*

The term 'schizophrenia' comes from the Greek and translates roughly as 'shattered mind'. Schizophrenia is a mental illness that is among the world's top ten causes of long-term disability affecting about 24 million people worldwide (World Health Organization (WHO), 2012). Thus, about 1% of the population is affected by schizophrenia, with similar rates across different countries, cultural groups and sex (Weiss and Feldon, 2001). The illness tends to develop between the ages of 16 and 30 years, and mostly persists throughout the patient's lifetime. Approximately 50% of discharged patients will be re-hospitalized within a year (Weiden *et al.*, 1996). Less than 20% of schizophrenia patients are employed at one time, 10% of patients will commit suicide after 10 years, while 15% will commit suicide in the ensuing 30 years after diagnosis (WHO, 2012). The majority of schizophrenia patients do not receive treatment, which contributes to the chronicity of the illness, while 20% of patients experience a relapse despite being on antipsychotic medication (WHO 2012; Fleischhacker and Hummer, 1997). In addition to severely disrupting the life of the patient and his/her family, schizophrenia incurs a great cost to society in terms of lost productivity and treatment-related expenses. Among psychiatric disorders, schizophrenia occupies about 25% of all psychiatric hospital beds (Terkelsen and Menikoff, 1995) and represents 50% of admissions to hospital (Geller *et al.*, 1991).

The primary manifestations of schizophrenia are an inability to filter incoming sensory information, disturbances in thinking, mood and overall behaviour (Eisendrath and Lichtmacher, 2005). Different combinations of symptoms with varying degrees of severity, as well as varying responses to antipsychotic treatment, are observed in schizophrenia patients, while the illness generally presents with poor long-term prognosis (Harvey *et al.*, 1999).

The heterogeneity of schizophrenia is often considered a major obstacle, involving profound disturbances of mental functions and subtle brain abnormalities that arise from a

combination of genetic, developmental and environmental factors (reviewed in Tseng *et al.*, 2009). While there is strong evidence for genetic transmission of vulnerability to schizophrenia (Harrison and Weinberger, 2005; Tsuang *et al.*, 2001), the heterogeneity and complexity of clinical phenotypes pose great obstacles for research into understanding the molecular and genetic basis of susceptibility for developing schizophrenia, indicating that other factors also contribute to the development of this devastating illness (Karayiorgou and Gogos, 1997, Horan *et al.*, 2008). Thus for example, neurotransmitter abnormalities, viral infections, stress, substance abuse, vitamin D deficiency, obstetric complications and altered immune function are all implicated in its pathogenesis (O'Brien *et al.*, 2008). Additionally, pre-, peri- and post-natal adversity, infection and vitamin D deficiency affecting neurodevelopment are notable risk factors in schizophrenia (McGrath *et al.*, 2010; Matheson *et al.*, 2011). Schizophrenia may therefore be a cluster of closely related diseases, further complicating our understanding of its pathophysiology. However, common pathophysiological pathways may exist while various brain regions and/or neural circuits may mediate differential expression patterns of the symptoms, as will be discussed below (Harrison *et al.*, 2005; Chambers *et al.*, 2001; Lipska and Weinberger, 2000).

2. Symptoms and clinical description

People diagnosed with schizophrenia usually experience a combination of symptoms that can be divided into four basic dimensions, viz. *negative*, *positive*, *cognitive* and *affective* (reviewed in Keshavan *et al.*, 2011), or unique domains of psychopathology presumably with a distinctive pathophysiology and treatment, as depicted in figure 1 (Tandon and Maj, 2008; Möller *et al.*, 2009). However, the pathophysiological validity of these dimensions has received strong support from clinical studies, demonstrating that each dimension has distinct cognitive, structural, metabolic and neurophysiological correlates. The number of relevant dimensions however remains an issue of debate (reviewed in Guillem *et al.*, 2005).

Positive symptoms can be described as reflecting an excess of normal function or being “psychotic”, while the negative symptoms are a loss of normal function or “psychomotor poverty” that severely disrupt the cognitive, intellectual and psychomotor functioning of the patient (Weiss and Feldon, 2001; Fuller *et al.*, 2003). Investigating the relationship between schizophrenia symptoms and personality, both in the acute phase of the illness and longitudinally may provide potentially important clues in understanding the pathophysiology of symptom expression (Guillem *et al.*, 2002). But let us first discuss the various symptoms domains of schizophrenia.

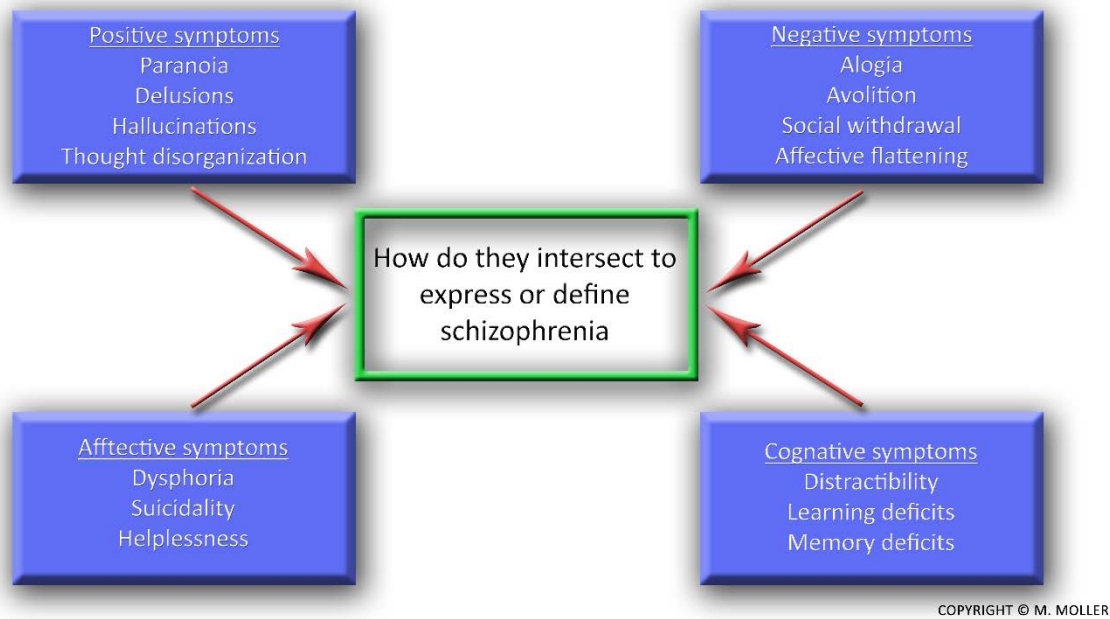


Figure 1: The four domains of schizophrenia symptoms: positive, negative, cognitive and affective symptoms (Adapted from Tandon and Maj, 2008; Möller *et al.*, 2009).

2.1 Positive symptoms

Positive symptoms involve two dimensions: a psychotic or reality distortion dimension (delusions and hallucinations), and a disorganization dimension (disorganized speech, thought disorders and inappropriate affect).

Delusions and hallucinations: Delusions can be defined as “firmly held erroneous beliefs” due to distortions or exaggerations of reasoning and/or misinterpretations of perceptions or experiences (Geyer and Vollenweider, 2008). Hallucinations are distortions or exaggerations of sensory perception, although auditory hallucinations (hearing voices, distinct from ones own thoughts) are the most common, followed by visual hallucinations (Mueser *et al.*, 2007).

Disorganized and catatonic behaviours: Grossly disorganized behaviour includes unpredictable agitation, difficulty in goal-directed behaviour, social dysfunction, or behaviours that are odd or inappropriate to society (DSM–V, American Psychiatric association, 2013). Catatonic behaviours are characterized by a marked decrease in reaction to the immediate surrounding environment, for e.g. motionless and apparent unawareness, rigid or bizarre postures, or aimless excessive motor activity.

Disorganized speech and thought: Disorganized speech or thinking, primarily based on the person's speech, is a very important presenting symptom of schizophrenia (reviewed in Subotnik *et al.*, 2006). Therefore, loosely associated or incoherent speech that is severe enough to substantially impair effective communication is used as an indicator of thought disorder (DSM–V, American Psychiatric association, 2013).

2.2 Negative symptoms

The negative symptoms primarily refer to the loss of motivation and emotional vibrancy (Lewis and Lieberman, 2000), including anhedonia, flat or blunted affect, poverty of speech (alogia), avolition (lack of initiative), and asociality (Andreasen and Olsen, 1982; Kay *et al.*, 1986). Negative symptoms are relatively common (Fenton and McGlashan, 1994) and are independent from positive, disorganized, and affective symptoms (Emsley *et al.*, 2003; Smith *et al.*, 1998). In addition, negative symptoms demonstrate unique associations with social functioning, neurocognition, and neurobiology (for a detailed review see Earnst and Kring, 1997). Since a range of causes can contribute to the expression of negative symptoms, it is important to distinguish between primary and secondary negative symptoms (Carpenter *et al.*, 1988; Kirkpatrick *et al.*, 2006). Primary negative symptoms are fundamental or intrinsic to schizophrenia, while secondary negative symptoms are caused by 'extrinsic' factors linked to schizophrenia, such as environmental deprivation, neuroleptic treatment and depression. The pathophysiology of negative symptoms is poorly understood (Keshavan *et al.*, 2008) and they remain relatively treatment-refractory as well as the most debilitating component of schizophrenia (Erhart *et al.*, 2006; Stahl and Buckley, 2007).

Affective flattening, alogia and avolition: Affective flattening is the reduction in the range and intensity of emotional expression, including facial expression, voice tone, eye contact, and body language (Kane *et al.*, 2009). Alogia, a deficit in speech fluency and productivity, is thought to resemble slow or inadequate thoughts, and often manifests as short, empty replies to questions (Iversen *et al.*, 2008). Avolition is the deficit or inability to persist in or initiate goal-directed behaviour (Iversen *et al.*, 2008).

Social withdrawal: Patients with schizophrenia are unable to integrate into society, while showing a marked lack of social interaction skills and social cognition (reviewed in Couture *et al.*, 2006), consequently impairment in social functioning represents a core behavioural feature of schizophrenia (Pinkham *et al.*, 2003), and are among the most debilitating and treatment refractory aspects of the illness (Bellack *et al.*, 2007).

2.3 Cognitive symptoms

Cognitive dysfunction has long been considered a primary characteristic of schizophrenia with many early clinical cognitive studies focusing on abnormal distractibility (Bergman *et al.*, 1995). Disturbances in basic cognitive functions, such as attention, executive functions and specific forms of memory (particularly working memory), are also consistently observed in patients with schizophrenia and are now thought to be central to the behavioural disturbances and functional disability of the disorder (Lewis and Lieberman, 2000). Cognitive rigidity is a common behaviour symptom of schizophrenia, for example these patients do not adapt normally to changes in their environments, especially in social and emotional contexts and they exhibit an inability to modify responses in formal testing situations (Bissonette and Powell, 2012). Another important cognitive dysfunction is impaired visual recognition memory (Calkins *et al.*, 2005). Here patients' perform poorly on many cognitive tasks such as the Wisconsin Card Sort Test (WCST) (Goldberg *et al.* 1987) and conditional associative learning paradigms (Gold *et al.* 2000).

2.4 Affective symptoms

It is well documented that patients with schizophrenia experience intense feelings of hopelessness, helplessness and a fragile sense of well-being. These symptoms are predictive of the persistence of psychosocial dysfunction and could contribute to suicidal ideation along with depression and anxiety (Lysaker *et al.*, 2001). Another symptom, dysphoria, includes both anxiety and depression and is associated with specific dimensions (positive and negative) of schizophrenia, with the exception of disorganization (Guillem *et al.*, 2005).

3. Diagnosis

The two most frequently used diagnostic classifications in psychiatry are the DSM-V American Psychiatric Association, 2013 and the ICD-11 World Health Organization, 2012. The diagnosis of schizophrenia requires at least 1-month duration of two (or more) of the following symptoms: (1) delusions, (2) hallucinations, (3) disorganized speech, (4) grossly abnormal psychomotor behaviour, including catatonia and (5) negative symptoms. At least one of these symptoms should include 1, 2, or 3. Further, one or more major areas of functioning, such as occupational or interpersonal social dysfunction, or self-care, should be markedly below the level achieved prior to the onset of symptoms for a period of at least 6

months (DSM–V, 2013). Exclusion criteria includes schizoaffective disorder, and depressive or bipolar disorder with psychotic features, as well as disturbances due to the direct physiological effects of a substance (e.g. an abused drug) or a general medical condition (DSM–V, American Psychiatric association, 2013).

4. Epidemiology and aetiology

With schizophrenia, both genetic and environmental risk factors need to be considered since both are important in the aetiology of schizophrenia and neither appears to operate in isolation (Tsuang *et al.*, 2004). Schizophrenia is highly heritable and genetic factors contribute to approximately 80% of the variability seen in the illness (Keshavan *et al.*, 2011). The distribution of a disease is generally expressed in terms of incidence (new cases) and prevalence, which refers to the total number of cases, existing and new (Tandon *et al.*, 2008). The estimated risk of developing schizophrenia over a lifetime ranges from 0.3–2.0% (Saha *et al.*, 2005), with an annual incidence of 8 - 40 per 100 000/year (Keshavan *et al.*, 2011). A meta-analysis of 24 studies found a median lifetime prevalence estimate for schizophrenia to be in the order of 4.0 per 1000 persons (Tandon *et al.*, 2008).

Schizophrenia aggregates in families, although over two-thirds of the cases occur sporadically. Nevertheless, having an affected family member substantially increases the risk of developing schizophrenia (Tandon *et al.*, 2008 for review). This risk increases as the degree of genetic affinity with the affected family member increases (Kendler and Diehl, 1993). Thus, if one monozygotic twin is afflicted with schizophrenia the other twin has a 50 - 70% risk of developing the illness as well (Goldberg *et al.*, 1995).

A variety of specific environmental exposures have been implicated in the aetiology of schizophrenia. These include both biological and psychosocial risk factors during the antenatal and perinatal periods, early and late childhood, adolescence and early adulthood (Maki *et al.*, 2005). In the antenatal period, maternal infections and nutritional deficiency, such as vitamin D (O'Brien *et al.*, 2008), during the first and early second trimesters of pregnancy are associated with an increased liability for developing schizophrenia (Penner and Brown, 2007; Meyer *et al.*, 2007). Exposure to infections, autoimmune, toxic or traumatic stress postnatally may also play a role in the pathogenesis of schizophrenia, perhaps via subtle alterations of neurodevelopment (Lewis and Lieberman, 2000). Thus, the aetiology of schizophrenia has been conceptualized as involving multiple hits (consisting of genes conferring vulnerability plus environmental insults), which are revealed in the context of developmental maturation of brain circuitry.

5. Pathophysiology

5.1 Neuroanatomy

Since the symptoms of schizophrenia are so divergent, it is difficult to relate a single brain structure or network to the behavioural and psychic aberrations of the illness (Fallon *et al.*, 2003). In an attempt to explain the brain circuitry involved in schizophrenia, an integrated neuroanatomical model has been put forward based on what is currently known about its neuroanatomy and chemistry (Lipska, 2004; Leonard 2003; figure 2 B), compared to the brain circuitry in healthy subjects (figure 2 A). This model places the primary deficit in the subcortical neurons projecting from the ventral tegmental area (VTA) to the cerebral cortex, postulating that a primary lesion, evoked by an unknown event before or after birth, later mediates a decreased activity of the PFC (figure 2 B). The latter is either due to neuronal atrophy or diminished neurogenesis, resulting in reduced neuronal connectivity in the PFC (Duman and Newton, 2007; figure 2 B). Prevailing evidence would now suggest that decreased PFC activity is expressed as hypo-function of critical dopamine (DA) -ergic and glutamatergic pathways (Coyle, 2006; Stahl *et al.*, 2007). Since the PFC is involved in the top-down control over activity of sub-cortical brain regions, the result is a weaker cortical feedback control on the VTA neurons and, simultaneously, in less effective cortical regulation of the limbic systems (LS), particularly the nucleus accumbens (NAcc). As a result, increased DAergic drive (from the partially disinhibited VTA neurons) acting on the NAcc, which at the same time is now less inhibited by the PFC (due to decreased glutamatergic activity), will allow greater VTA-directed stimulation of the NAcc (figure 2 B). Increased (disinhibited) DAergic activity projecting from the VTA is now less effective in driving the activity of PFC under such conditions, especially in lieu of the existing primary glutamatergic (excitatory) deficiency (figure 2 B).

Although useful conceptually, this model may require further modification and refinement to account for additional characteristics of schizophrenia, such as the time course of the illness or the role of stressful events in triggering the disease (Holcomb *et al.*, 2004; Moghaddam, 2002). However, the salient feature of the model, viz. DAergic and glutamatergic deficits in the PFC upstream from hyper-dopaminergic activity in the LS (Holcomb *et al.*, 2004), has important construct and heuristic value in explaining both the positive (hyper-active LS; figure 2 B) and negative symptoms, as well as the cognitive deficits, of schizophrenia. These deficits are known to be accompanied by a reduced activity in the PFC in patients with schizophrenia, as well as in associated brain structures such as the mediodorsal nucleus of the thalamus (Yang *et al.*, 2003; Lehrer *et al.*, 2005), and that drive the fragmentation of

cognitive processing. Central executive function, especially the manipulation of transiently stored information is also disturbed in schizophrenia (Cannon *et al.*, 2005) and accompanied by altered activation of the PFC (Callicott *et al.*, 2003).

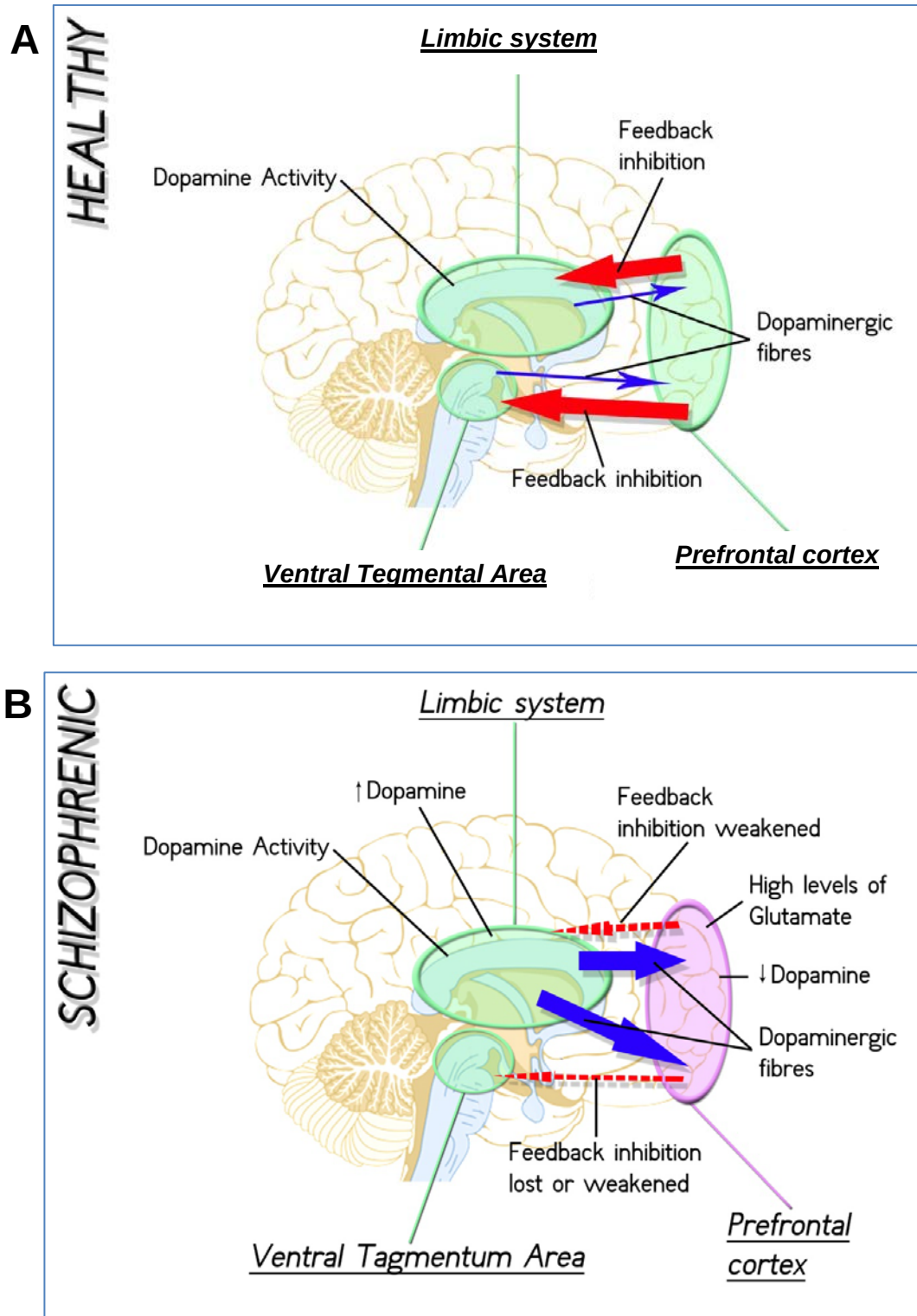


Figure 2: The brain circuits involved in schizophrenia in (A) healthy subjects, compared to (B) schizophrenia patients (Adapted Leonard, 2003; Möller *et al.*, 2009). Refer to the text for detailed explanation.

The most frequent neurobiological finding in schizophrenia is enlargement of the ventricular system as well as a smaller hippocampus, and thalamus (Sawa and Snyder, 2002; Wright *et al.*, 2000; MacDonald and Schulz, 2009). The latter two brain areas participate in emotional regulation and cognitive functions, processes that are impaired in schizophrenia (discussed in section 2). Ventricular enlargement is accompanied by overall reductions in brain volume and cortical grey matter (Goldman *et al.*, 2008). Magnetic resonance imaging (MRI) studies have found reduced grey matter in schizophrenia patients compared to healthy controls, in specifically the prefrontal cortex (Sigmundsson *et al.*, 2001; Thompson *et al.*, 2001), as indicated in figure 3. This illustration reveals significant, progressive gray matter loss in schizophrenia patients over time (as indicated on the right side of figure 3). Progressive loss occurs in schizophrenia in parietal, motor, supplementary motor, and superior frontal cortices, while broad regions of the temporal cortex, including the superior temporal gyrus, experience severe loss of gray matter (figure 3; Thompson *et al.*, 2001). Dynamic loss is also observed in the parietal cortices of normal adolescents, but at a much slower rate (Thompson *et al.*, 2001). However, a recent study indicated that fronto-temporal brain structural abnormalities are evident in nonpsychotic individuals at high risk of developing schizophrenia, and that these gray matter abnormalities become more extensive from first-episode through to chronic schizophrenia (Chan *et al.*, 2011). Mapping the progressive changes in schizophrenia, from shared genetic factors through to chronic illness, clarifies potential markers for disease risk (e.g. anterior cingulate and right insula volume reduction), early onset (e.g. caudate volume reduction) and progression to chronic stages (e.g. thalamic involvement) (Thompson *et al.*, 2001; Wood *et al.*, 2008; Theberge *et al.*, 2003).

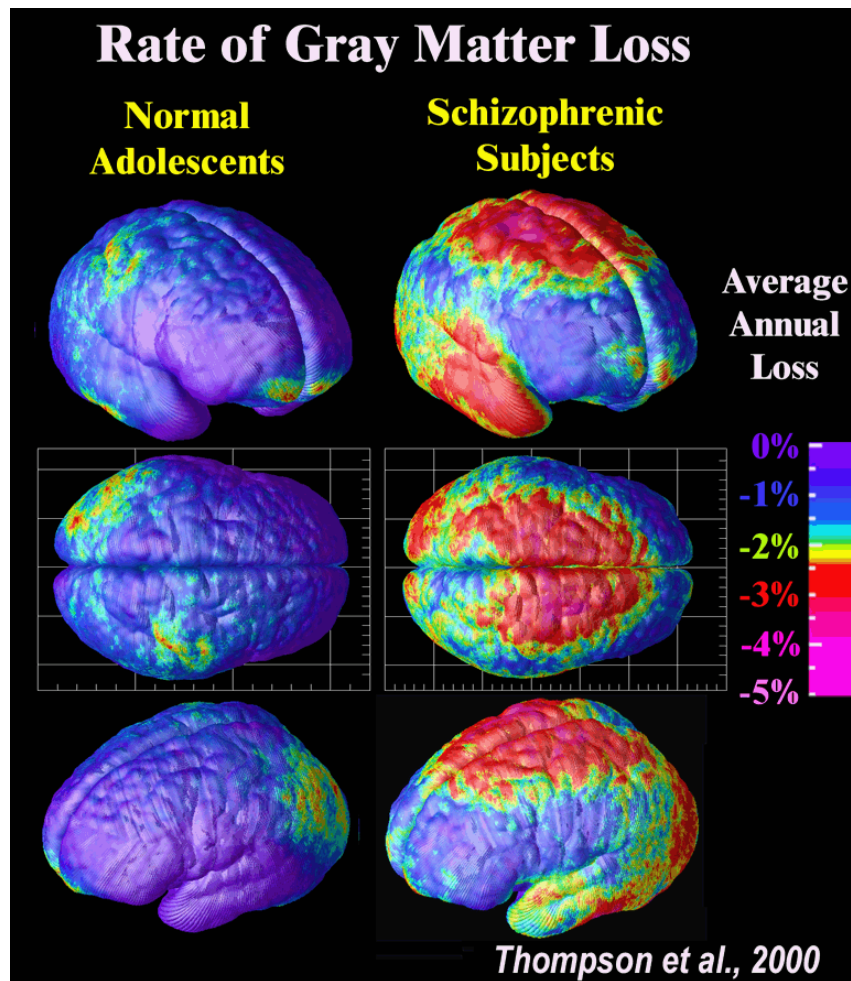


Figure 3: Three-dimensional maps of brain changes over time using high-resolution MRI scans acquired from the same group of subjects and at the same age, showing dynamic gray matter loss in normal adolescents and in patients with schizophrenia. The average rate of gray matter loss from 13 to 18 years of age is displayed in both schizophrenia patients and healthy controls. This severe loss is observed (red and pink; up to 5% annually) in the parietal, motor, and temporal cortices, whereas inferior frontal cortices remain stable (blue; 0–1% loss) (Thompson *et al.*, 2001).

Abnormalities in cerebral blood flow (CBF) have also been shown in the frontal regions, thalamus and cerebellum of schizophrenia patients in positron emission tomography (PET) studies (Andreasen *et al.*, 1996). This hypo-frontality with respect to blood flow can be linked to diminished DA activity and therefore decreased cognitive functioning, as is observed in the pathophysiology of schizophrenia (Mueser and McGurk, 2004). A recent study that measured CBF with arterial spin labeling (ASL) perfusion MRI noted decreased CBF in the bilateral precuneus and middle frontal gyrus in patients with schizophrenia as well as an increase in CBF in left putamen/superior corona radiata and right middle temporal gyrus (Pinkham *et al.*, 2011). A decrease (23%) in the dendritic spine density on the hippocampus and the medial part of the prefrontal cortical pyramidal neurons is also a distinct feature in schizophrenia patients compared to healthy controls (Glantz and Lewis, 2000).

The neuroanatomical development of schizophrenia therefore appears to have a direct relationship with the deficits shown in imaging data of specifically the subcortical regions (nucleus accumbens and hippocampus) and the PFC in schizophrenia patients (Weiss and Feldon, 2001; Shad *et al.*, 2006). But what is the basis for the initial lesion in early development, as well as the mechanisms underlying the progressive degeneration of these brain regions post diagnosis? This is discussed in the following section, and indeed is the focus of this study.

5.2 Neurodevelopmental anatomy

Adverse events experienced in early life may contribute to the expression or exacerbation of a variety of physical and psychological disorders, and is particularly valuable for our understanding of schizophrenia (Lipska and Weinberger, 2000). Weinberger (1986) and Murray and Lewis (1987) first formulated the “neurodevelopmental hypothesis of schizophrenia” stating that abnormalities of early brain development increase the risk for subsequent emergence of clinical symptoms (Marenco *et al.*, 2002). All the regions of the human brain are formed prenatally; however neurodevelopment extends throughout the life span (Walker *et al.*, 2008). Since schizophrenia does not develop acutely, but through a gradual prodromal phase that takes place over a prolonged period (months to several years), it may be important to intervene early on in the developmental phase of the illness and to identify pivotal neurobiological markers that drive the pathophysiology of schizophrenia.

The initial prodrome of schizophrenia describes a period of time that begins with the first visible changes in the person and extends up to the development of the first psychotic episode (Yung and McGorry, 1996). Prodromal symptoms include depression, anxiety, and decreased social interaction, but the key factors defining the prodrome are attenuated psychotic symptoms (perceptual abnormalities, unusual ideations, disturbances in thought and suspiciousness) (Walker *et al.*, 2010). Early and late neurodevelopmental disturbances in schizophrenia and their functional consequences involve structural brain abnormalities that may already be apparent in the prodromal stage, which usually has its onset during adolescence (Pantelis *et al.*, 2003). Indeed, MRI imaging and novel neuroanatomical marker studies all agree that schizophrenia may be a neurodevelopmental disorder (Sawa and Snyder, 2002).

Underlying the macroscopic changes in schizophrenia, two very important histological alterations are noted (Arnold and Trojanowski, 1996). Firstly, the cortical cytoarchitecture is altered, with neurons being misplaced, abnormally sized, and disorganized (Harrison, 1997).

These abnormalities are highly indicative of an early developmental origin with an onset no later than infancy. Secondly, the neurodegenerative outline of schizophrenia in the absence of glial reactions (Weinberger and Marenco, 2003) confirms that the neuropathological changes in schizophrenia are prenatal rather than postnatal. Glial reactions are associated with most adult-onset brain injuries, as well as with neurodegenerative disorders such as Alzheimer's and Parkinson's disease, and are not found in neurodegenerative disorders that arise during early brain development (Weinberger and Marenco, 2003). In addition, neuropsychological studies in children also support an early onset brain abnormality that later leads to the development of schizophrenia. These children present with distinct neuromotor, neuropsychological and intellectual abnormalities in early childhood, even before any psychiatric symptoms appear (Done *et al.*, 1994; Cannon *et al.*, 1994). However, the question remains as to why schizophrenia emerges during late adolescence? In a review by Uhlhaas (2011), the explanation relies on findings that brain development during adolescence serves as a trigger for the expression of psychosis via the following pathways: (1) excess pruning of synaptic contacts during adolescence that leads to reductions in grey matter volume (Gogtay *et al.*, 2011); (2) the possible role of aberrant maturation of excitatory glutamatergic circuits during adolescence along with the refinements in γ -amino butyric acid (GABA) receptors (Hoftman and Lewis 2011); and (3) hormonal changes during adolescence that affects brain function and development (Walker *et al.*, 2010).

Another important finding is that brain asymmetry (specifically lack of normal hemispheric volume asymmetries) is reduced in schizophrenia (Bilder *et al.*, 1994). A developmental origin is the most plausible explanation for this, given the normally asymmetrical growth of the cerebral hemispheres (Bakalar *et al.*, 2009). Abnormal low levels of neuropil, abnormalities in synaptic, dendritic, axonal and white matter tract organization and abnormal glutamatergic neurotransmission (Coyle, 1996; Zaidel *et al.*, 1997), may also indicate the neurodevelopmental time line in the brain of the schizophrenia patient, which are consistent with defective connectivity between brain regions such as the midbrain, nucleus accumbens, thalamus, temporo-limbic and prefrontal cortices (Arnold, 1999; Selemon and Goldman-Rakic, 1999).

The neurodevelopmental aetiology of schizophrenia links genetic risk to environmental risk factors such as perinatal insults (Coyle and Tsai, 2004), as described in section 4. For e.g., when an individual has inherited one or more genes that code for abnormal proteins, and these proteins likely modify the way the mesolimbic DA pathway operates, these insults may ultimately lead to congenital vulnerability of the DA circuitry and GABAergic neuronal damage (figure 4 & 5) (Schwartz *et al.*, 2012 for review). Evidence of which being reduced

expression of presynaptic markers in subpopulations of GABAergic interneurons in the frontal cortex and the hippocampal formation of schizophrenia patients (Lewis and Frangou, 2003 for review). These GABAergic neurons play an important role in regulating the activity of the projecting glutamatergic pyramidal cells, illustrated in figure 4 (Benes and Berretta, 2001).

DA neuronal activity originating in the midbrain is controlled by primary glutamate pyramidal neurons in the PFC that activate N-methyl-D-aspartate (NMDA) receptors on GABA interneurons (figure 4). These GABA interneurons in turn synapse with secondary cortical pyramidal glutamate neurons responsible for down-stream neurotransmitter (DA, NA, 5HT) release in the striatum, raphe nucleus, locus coeruleus and ventral tegmental area (Schwartz *et al.*, 2012; figure 4, left panel). In schizophrenia, genetic and environmental risk factors may cause defective, insensitive secondary cortical NMDA receptors, and via the above described glutamate-GABA-glutamate loop, will prompt excessive DA release in the VTA and LS, ultimately responsible for the genesis of psychotic and positive symptoms (figure 4 left panel) (Schwartz *et al.*, 2012; Carlsson *et al.*, 2001).

Regarding the negative symptoms of schizophrenia (figure 4, right panel), the cortical brainstem glutamate projection has two series of GABA interneurons, one of which impacts on midbrain VTA DA neurons. These mesocortical DA neurons ascend back to the dorsolateral prefrontal cortex (DLPFC) and ventromedial prefrontal cortex (VMPFC) with the purpose of providing sufficient activity for alertness, concentration, emotional and executive functioning (Schwartz *et al.*, 2012). As with the defect in the cortical glutamate-GABA-glutamate loop described above for positive symptom development, the secondary glutamate neuron is again hyper-active (figure 4, right panel). However in this scenario the secondary glutamate neuron impinges on this other GABA interneuron that is now stimulated by the high glutamate tone to release GABA. The ensuing increase in GABA inhibits VTA DA neurons resulting in less DA mesocortical activity, eventually causing hypo-frontality and negative symptoms (figure 4, right panel) (Schwartz *et al.*, 2012). Glutamate is critically involved in neuronal development, neuroplasticity, neurotoxicity and formation of synapses (Goff and Coyle, 2001; see Konradi and Heckers, 2003 for review).

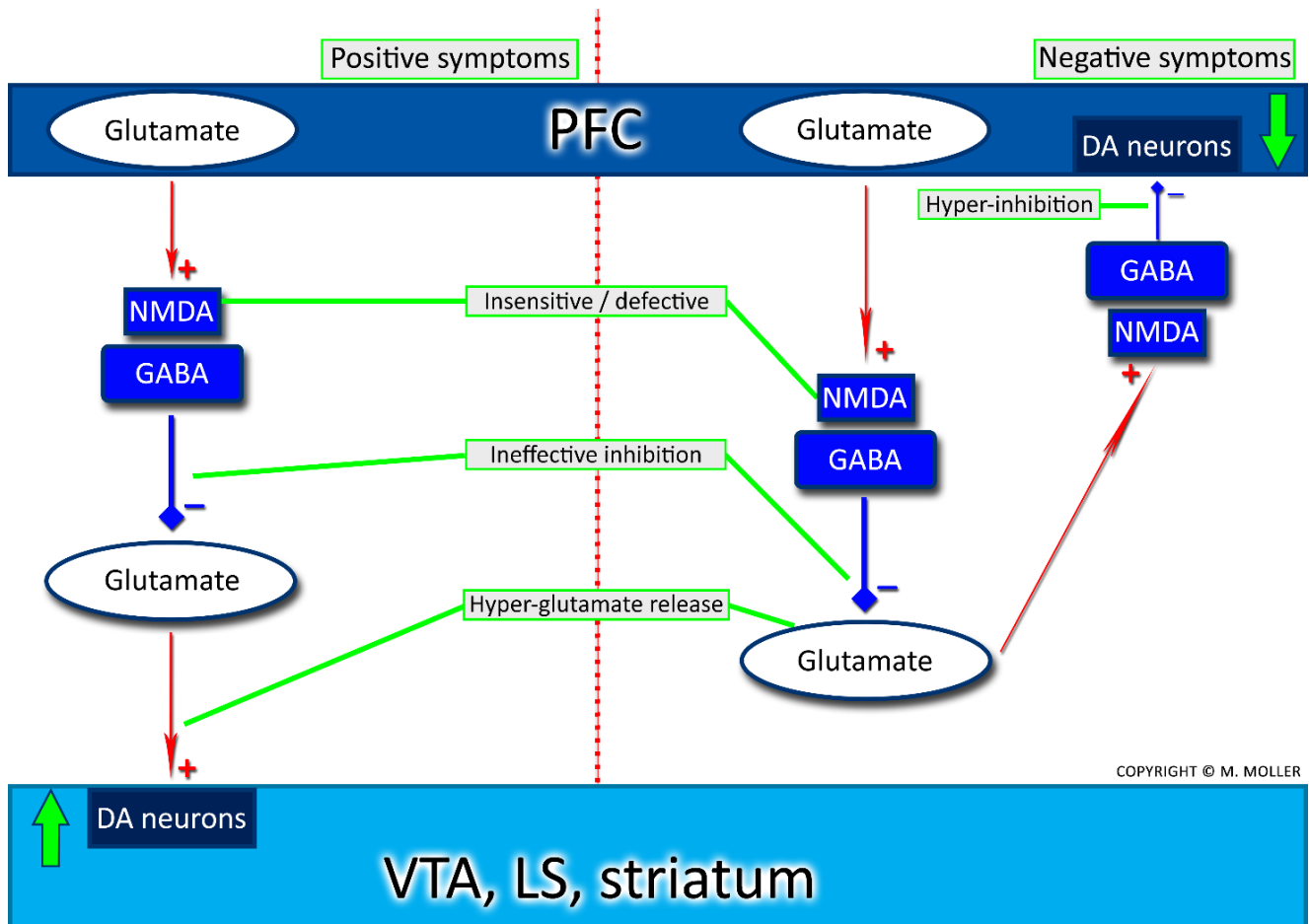


Figure 4: A simplified diagram depicting the neurocircuits involved in positive (left panel) and negative (right panel) symptoms observed in schizophrenia due to neuronal developmental abnormalities (Modification from: Schwartz *et al.*, 2012; Carlsson *et al.*, 2001).

Reduced levels of glutamate (figure 5) or hypo-activity at NMDA receptors (figure 4), will ultimately impact on the number of synapses established, resulting in abnormalities in brain development, brain circuitry and deficient synaptic connectivity, all linked to the neurodevelopmental theory of schizophrenia (Lewis and Lieberman, 2000). Goff and Coyle (2001) also suggests that a primary hypo-active glutamate system in schizophrenia could influence the formation of neuronal connections in the cortical and subcortical brain areas early in life, which fits well with the anatomical abnormalities found in the adult schizophrenia brain. Conversely, the glutamate system can also be inhibited by DA, or be facilitated by the inhibition of D₂ receptors (Konradi and Heckers, 2003 for review) and is strongly implicated in the neurochemistry of schizophrenia (see section 4.3.). Continuing to the right in figure 5, it is not until later in life, following onset of adolescent maturation, that vulnerability begins to be manifested in the prodromal signs of psychosis. This neuropathological model (figure 5) also proposes that activation of the hypothalamic-pituitary-gonadal (HPG) axis during adolescence, as well as hypothalamic-pituitary-adrenal (HPA) hyper-activity and hyper-

cortisolemia may trigger both DA activity and gene expression changes, contributing to the neurodegenerative changes that results in cognitive, social and emotional dysfunction leading to the first episode of psychosis during young adulthood (Walker *et al.*, 2010).

Concluding, the neurodevelopmental hypothesis suggests that brain development can be adversely affected at a critical time of life (figure 5), particularly through early life exposures to stress that may provoke the onset of psychosis in later adolescence or adulthood (Weiss and Feldon, 2001; Walker *et al.*, 2010), along with associated neurochemical changes (summarized in figure 5). Increased (sub-cortical) and decreased (cortical) glutamate, reduced cortical GABA and increased (sub-cortical) and decreased (cortical) DA are all central to this hypothesis. However, other mechanisms and hypothesis are likely to be involved (explained in section 5.5 – 5.7).

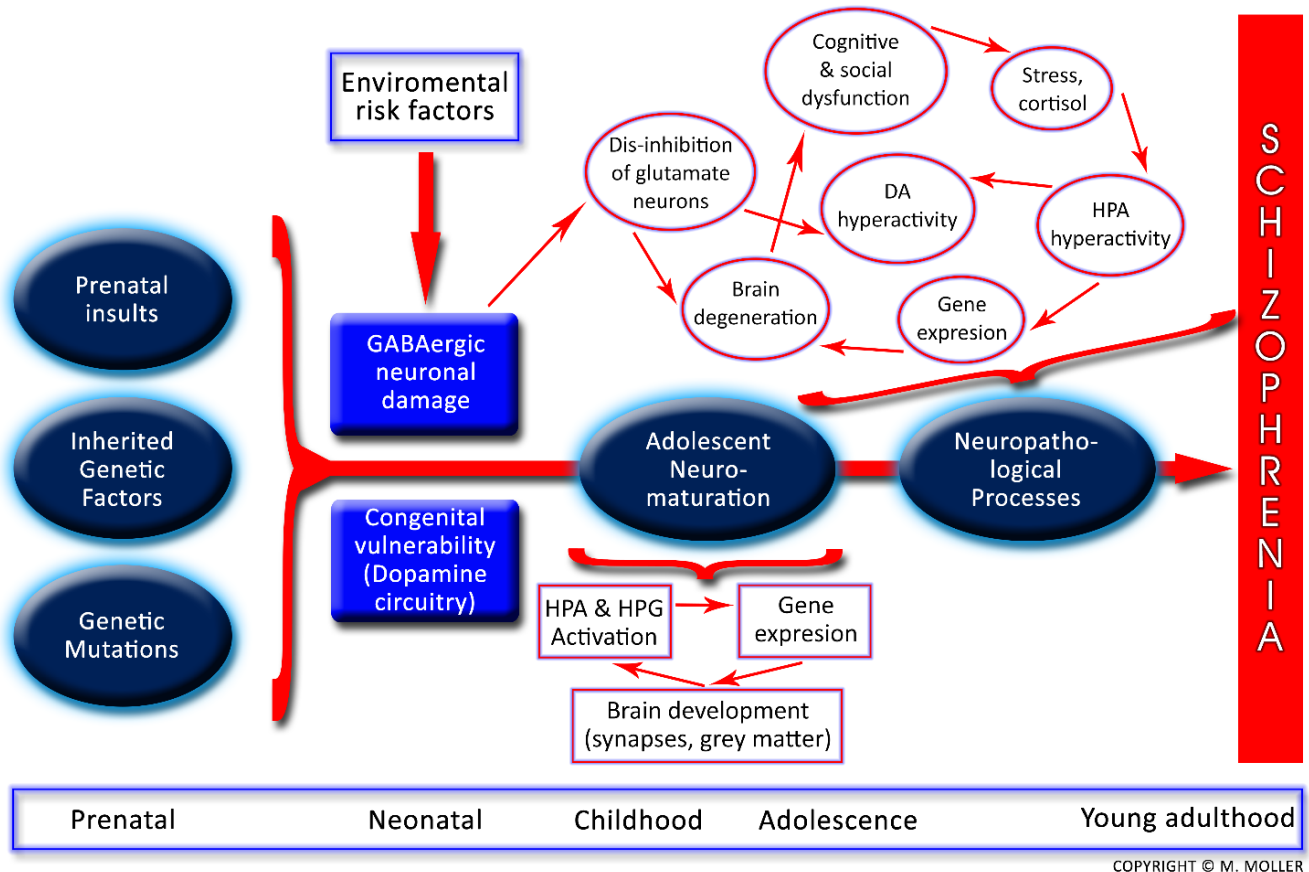


Figure 5: A depiction of the neurodevelopmental pathogenesis of schizophrenia, with onset of psychosis and how various developmental and neuropathological processes are involved (Adapted from: Reynolds, 2005 and Walker *et al.*, 2010).

5.3 Neurochemistry

Running concurrently with the neurodevelopmental hypothesis of schizophrenia (figure 5) is the dysfunction of a number of neurotransmitter systems. This has formed the principal construct dominating neuropharmacological research into new drug development in schizophrenia. The following hypotheses have been developed to evaluate the extent to which neurochemical findings reflect primary or secondary mechanisms involved in the illness. Today these theories form the basis for explaining the mode of action of all currently used drugs for the treatment of schizophrenia, and in many ways still determine the way forward for new drug development.

5.3.1 The Dopamine hypothesis

The classical “dopamine hypothesis of schizophrenia” postulates a hyper-activity of DAergic transmission at the D₂ receptor, specifically in the mesolimbic projections to the LS (Carlsson, 1988). This hypothesis was first based on the ability of DA agonists, for e.g. amphetamines which stimulate DA release, to induce psychosis with schizophrenia-like features in healthy subjects, and at very low doses to provoke psychotic features in schizophrenia patients (Miyamoto *et al.*, 2003). In animals, amphetamine is used in the DA sensitization model of schizophrenia (Tenn *et al.*, 2003 for review). This notion was also supported by the correlation between the therapeutic doses of conventional antipsychotics and their affinities for the D₂ subtype(s) of DA receptors (Miyamoto *et al.*, 2001). Subsequently, the DA hypothesis has received strong support from PET studies, indicating a higher density of D₂ receptors in post-mortem brain in patients with schizophrenia (Wong *et al.*, 1986), as well as imaging studies indicating the close correlation between D₂ receptor binding and efficacy of these drugs to decrease psychosis (Corripio *et al.*, 2005; Carlsson *et al.*, 1997 for review). This work led to the formulation of modified DA hypotheses in which elevated D₂ receptors were proposed to underlie the positive symptoms of schizophrenia (Reynolds, 2005) and that there is an imbalance between subcortical and cortical DA levels (Duncan *et al.*, 1999; Tzschentke, 2001).

Numerous studies have revised the DA hypothesis to include the cortical and subcortical components of the brain (Grace, 1991; Davis *et al.*, 1991). Evidence that patients with schizophrenia have higher levels of DA has also been found in the striatum at post mortem (Guillin *et al.*, 2007a). A hyper-activity of DA prevails in the mesolimbic DA projections and in the DA cell bodies located in the VTA, resulting in hyper-stimulation of D₂ receptors and ultimately causing psychotic, positive symptoms. On the other hand, a hypo-dopaminergic state, caused by mesocortical hypo-active DA projections, is observed in the frontal cortical terminal fields, resulting in the negative symptoms of the illness (Guillin *et al.*, 2007a, b).

Despite the importance and relevance of the DA hypothesis in explaining the neurobiology and pharmacology of schizophrenia, there are still noteworthy limitations that need to be considered.

Firstly, there is no framework describing how striatal hyper-dopaminergia translates into positive symptoms (delusions) or how frontal hypo-dopaminergia translates into negative symptoms (social withdrawal) (Howes and Kapur *et al.*, 2009). This is mainly due to the fact that presynaptic DA function in the frontal cortex is not at present accessible to noninvasive imaging studies (Carlsson *et al.*, 1988; Guillin *et al.*, 2007a). Secondly, no differences in the percentage of D₂ receptor occupancy has been found in responders compared to non-responders to antipsychotic treatment (Coppens *et al.*, 1991). Furthermore, only 30% of patients with schizophrenia respond to typical D₂ receptor antagonists (Chavez-Noriega *et al.*, 2002). Thirdly, the development of low potency atypical antipsychotics such as clozapine and quetiapine, demonstrate exceptionally low affinity for D₂ receptors in relation to their therapeutic dose (Kerwin and Dumon, 1994; Harvey *et al.*, 1999). When clozapine reaches its therapeutic dose in plasma, only 30-60% of D₂ receptors are occupied, whereas 80-90% of 5-HT₂ receptors are occupied (Farde *et al.*, 1992; Nyberg *et al.*, 1996). Later on, clozapine was found to be a more potent blocker of the D₄ receptor (Harvey *et al.*, 1999; Burstein *et al.*, 2005). Furthermore, the introduction of new atypical antipsychotics similar to clozapine emphasized the important role of serotonin (5-HT), but also a host of other signaling pathways, such as the cholinergic and adrenergic systems (see Harvey *et al.*, 1999 for review), which together began to question the immediate importance of D₂ receptor binding for adequate antipsychotic action. However, later evidence that D₄ blockers are ineffective antipsychotics (Kramer *et al.*, 1997), and that D₂ blockade is necessary and sufficient for antipsychotic efficacy (Kapur and Ramington, 2001), re-affirmed the important role of DA in the neurobiology of schizophrenia, and indeed of the role of the D₂ receptor in antipsychotic drug action.

In an effort to re-evaluate the DA hypothesis, new evidence in schizophrenia patients was recently reviewed by Howes and Kapur 2009. These authors came to the following conclusions:

- PET scanning found elevated presynaptic levels of DA in the striatum.
- Baseline occupancy of D₂ receptors by DA is increased.
- DAergic transmission in the PFC is mainly mediated by D₁ receptors, and D₁ dysfunction has been linked to cognitive impairment and negative symptoms of schizophrenia.
- Genes in combination with adverse environmental factors affects the DA system.

- GABA interneurons are important in the regulation of subcortical DA.

Howes and Kapur (2009) therefore proposed that multiple “hits” (i.e. adverse environment) interact to result in DA dysregulation, the final common pathway to psychosis in schizophrenia. The exact diagnosis, however, reflects the nature of the hits coupled with sociocultural factors and not the DA dysfunction per se.

While the role of DA cannot be disregarded, given the limitations of the hypothesis noted earlier, it is clear that pharmacological properties other than D₂ receptor antagonism may contribute to a more effective management of schizophrenia. Schizophrenia represents DA dysregulation in the context of a compromised brain, so that future drug development should therefore focus on new pathways and mechanism that impact directly or indirectly on said DA dysregulation (Kapur, 2003; Stone *et al.*, 2007).

5.3.2 Serotonin hypothesis

Current thinking supports the view that other neurotransmitters such as 5-HT might also contribute to the aetiology of schizophrenia. Indeed, research on the serotonergic system has been gaining attention and importance since the availability of clozapine and the realisation of its improved efficacy for negative symptoms and markedly reduced side effect profile (Harvey *et al.*, 1999; Chakos *et al.*, 2001). Clozapine is a multipotent antagonist, but in particular has a low affinity for the D₂ receptor, coupled with a high affinity for the 5HT₂ receptor (See section 6.2; Harvey *et al.*, 1999; Sanyal and Van Tol, 1997).

The first hypotheses concerning the involvement of 5-HT in schizophrenia was advanced by Woolley and Shaw (1957) and Gaddum and Hammeed (1954), and was based on the psychotomimetic effects of lysergic acid diethylamide (LSD). LSD is structurally related to 5-HT, and was proposed to be an antagonist at brain 5-HT receptors. However, an inherent drawback to this hypothesis is that the primary effect of LSD is to produce visual hallucinations, which are relatively rare in schizophrenia, and not auditory hallucinations which are the most common perceptual disturbance in schizophrenia (Aghajanian and Marek, 2000). Another concern with this hypothesis is that LSD is a full or partial agonist at many 5-HT receptors rather than an antagonist, as originally predicted (Shaw and Woolley, 1957 as reviewed by Aghajanian and Marek, 2000). Since the drug is a powerful hallucinogen, causing psychotic symptoms in healthy subjects, investigators proposed that serotonergic activity might be decreased in schizophrenia. Indeed, this has credence since 5-HT_{2A} and 5-HT_{1A} receptors are altered in cortical brain areas of schizophrenia patients (Harrison, 1999; Lieberman *et al.*, 1998), along with reduced 5-HT_{2A}-receptor density in the frontal cortex of drug naïve schizophrenia patients (Hurlmann, 2008). However, the

psychotic symptoms induced by glutamate NMDA receptor antagonists are blocked by atypical antipsychotics (e.g., clozapine) and selective 5-HT_{2A} antagonists, thus contradicting the earlier mentioned theory. Indeed, both hallucinogens and NMDA antagonists (e.g. ketamine) enhance glutamatergic transmission via stimulation of 5-HT_{2A} receptors (Aghajanian and Marek, 2000). These findings not only put into perspective the role of 5HT in psychosis, but emphasises that the primary mediator of psychosis is the glutamatergic system. Furthermore, clinical studies using selective 5HT_{2A/2C} antagonists are ineffective as antipsychotics (reviewed in Roth *et al.*, 2004). Nevertheless, post-mortem-studies, as well as the examination of 5-HT in the cerebrospinal fluid, genetic studies and neuroimaging findings, have demonstrated an increase in central serotonergic neurotransmission in schizophrenia (Harrison, 1999; Ngan and Liddle, 2000; Eastwood and Harrison, 2001; Dean, 2003). One might therefore speculate that the early phases of schizophrenia are dominated by neurobiological abnormalities involving 5-HT receptors, but that the subsequent course of the disease involves more complex functional alterations in the serotonergic system (including both pre- and postsynaptic function) affecting multiple neurotransmitter systems (e.g., glutamate, GABA, noradrenaline (NA), acetylcholine, and DA) and therefore contributes to the various behavioural disturbances in schizophrenia (Fallon *et al.*, 2003; Geyer and Vollenweider, 2008).

5.3.3 Glutamate and gamma-aminobutyric acid (GABA) hypothesis:

GABA and glutamate are respectively the most common inhibitory and excitatory neurotransmitters in the brain, so that a disturbance in glutamate and/or GABA in schizophrenia are an important consideration. Indeed, as alluded to earlier, psychosis is evoked by the administration of antagonists of the NMDA receptor such as phencyclidine (PCP) and ketamine, both non-competitive NMDA antagonists (Wang *et al.*, 2007 for review). Moreover, the psychogenic effects of these drugs mimic that of schizophrenia, including negative symptoms, positive symptoms and cognitive deficits (Abi-Saab *et al.*, 1998), thus providing a closer representation of the overall symptoms of schizophrenia (Reynolds, 2005; Adler *et al.*, 1998; Krystal *et al.*, 1994). However, overstimulation of the glutamatergic system can provoke hyper-excitability, pro-convulsant activity and neuronal damage (Meldrum, 2000). At a cellular level glutamate has a strong influence in controlling neurogenesis and neuroplasticity (Spedding *et al.*, 2003).

It has been suggested that a predisposing factor in schizophrenia may involve a decrease in cortical NMDA receptor function (Javitt and Zukin, 1991), specifically NMDA receptor hypo-function, that precipitates the cognitive and negative symptoms observed in schizophrenia, discussed in section 5.2 and illustrated in figure 4, right panel (Schwartz *et al.*, 2012; Stahl,

2007; Carlsson *et al.*, 2000). Glutamatergic hypo-function in frontal-cortical areas is therefore seen as the initiating factor evoking a reactive increase in DAergic function in limbic brain regions (see figure 4 left panel and 5) (Holcomb *et al.*, 2004). Confirming this, partial deletion of the NMDA receptor 1 subunit in mice is associated with behavioural alterations akin to that observed in PCP treated mice (Mohn *et al.*, 1999). In post-mortem studies of schizophrenia, deficits of glutamate systems have been described in the temporal cortex, medial temporal lobe and striatal regions (Bauer *et al.*, 2008; Goff and Coyle, 2001), with losses of glutamate uptake sites (Aparicio-Legarza *et al.*, 1997) and increases in NMDA receptors (Nudmamud-Thanoi and Reynolds, 2004). A recent clinical study also indicated elevated GABA and glutamate levels in medial PFC of unmedicated patients, with no alterations in medicated schizophrenia patients, suggesting possible normalization of GABA and glutamate with antipsychotic medication (Kegeles *et al.*, 2012). Similar changes have been observed in animal models of schizophrenia, with decreased glutamate release in the frontal cortex of the Homer1 mutant mice (Szumlinski *et al.*, 2005), while chronic PCP administration in rats is associated with a decreased expression of glutamate receptors in the PFC (Barbon *et al.*, 2007). In a previous study we demonstrated an increase in NMDA receptor binding in the frontal cortex in animals subjected to isolation rearing, a putative model of schizophrenia (see section 8.2) (Toua *et al.*, 2010). Glutamate stimulation of NMDA receptors also activates a number of sub-cellular messengers such as nitric oxide synthase (NOS), involved in oxidative stress, and nuclear factor- $\kappa\beta$, involved in inflammatory responses (Oosthuizen *et al.*, 2005), both messengers being altered in schizophrenia (discussed in section 5.4 and 5.5 respectively).

Interestingly, changes in glutamate is thought to be mediated by a hypo-function of cortical GABAergic neurons on which NMDA receptors are located, resulting in disinhibiting and hence over activity of downstream glutamatergic neurons (discussed in section 5.2, figure 4; Schwartz *et al.*, 2012), with evidence of significant losses in cortical and hippocampal GABA-containing neurons (Fallon *et al.*, 2003; Lewis and Lieberman, 2000). Glutamate interneurons synapse on other neuronal systems to influence down-stream signaling, for e.g. NA, DA and 5-HT, such that dysfunction in GABA/glutamate transmission may be a significant contributor to 5-HT and DA dysfunction in schizophrenia (Harvey *et al.*, 1999), discussed in more detail in section 5.2. NMDA receptor function is also strongly influenced by the kynurenine pathway and that has relevance in schizophrenia (Stone and Darlington 2002, discussed in section 5.5).

The above discussion emphasizes that schizophrenia is a severe, disabling disorder with multiple neurotransmitter dysfunctions. However, the role of oxidative stress, tryptophan metabolism via the kynurenine pathway, inflammation and mitochondrial imbalance in schizophrenia is becoming increasingly relevant and interacts at various levels with the above-mentioned transmitter networks. This will be discussed in the following sections.

5.4 Oxidative stress

Converging evidence indicates that schizophrenia is a neurodevelopmental disorder (section 4.2), while various anatomical findings point to a vastly distributed neuropathology, possibly involving oxidative stress (Do *et al.*, 2000). Oxidative stress occurs when cellular antioxidant defence mechanisms (superoxide dismutase (SOD), catalase, glutathione peroxidase) fail to counterbalance and control endogenous reactive oxygen species (ROS) such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2) generated from normal oxidative metabolism or from pro-oxidant environmental exposures (Bitanhirwe and Woo, 2011). Mitochondria are the major source of ROS that in turn are quenched by SOD, catalase and the glutathione system (Bains and Shaw, 1997; Johnson and Giulvi, 2005). Importantly, mitochondrial diseases may be associated with secondary neurotransmitter disturbances that may mediate an assortment of effects associated with schizophrenia (Garcia-Cazorla *et al.*, 2008). SOD is the primary defense against oxidative stress by converting O_2^- to H_2O_2 (Bains and Shaw, 1997). Hydrogen peroxide in turn is converted to water and the oxidized (disulphide) form of glutathione (GSSG) by catalase and glutathione peroxidase (Griffith, 1999), the latter rapidly being converted back to reduced glutathione (GSH) by glutathione reductase (Bouligand *et al.*, 2006). A reduction in GSH, and an increase in GSSG, is regarded as being indicative of increased oxidative stress.

The brain is particularly vulnerable to oxidative damage (McQuillen and Ferriero, 2004), given its relatively low content of antioxidant defenses in addition to its high metal content, which can catalyse the formation of ROS (reviewed in Bitanhirwe and Woo, 2011). The neurodevelopment of schizophrenia is believed to be propagated by early environmental insults that result in an increase in ROS, lipid and protein peroxidation and DNA damage, and a decrease in GSH and antioxidant defence systems (Akyol, 2002a, and b). Early life insults also lead to increased inflammation (Garcia-Bueno *et al.*, 2005) emphasizing the close link between inflammation, oxidative stress and the neurodevelopmental hypothesis of schizophrenia. The developmental dysregulation of GSH synthesis in schizophrenia is proposed to be of genetic origin (Do *et al.*, 2009) and, when combined with environmental

risk factors that can boost levels of oxidative stress, may play a critical role in inducing deficits in neural connectivity and synchronization evident in the disease (Do *et al.*, 2009).

Evidence has accumulated in recent years that antioxidant systems are impaired in schizophrenia (Mahadik and Mukherjee, 1996). Gawryluk *et al.* (2010) also reported reduced levels of GSH in post-mortem PFC of patients with schizophrenia. Do and colleagues (2000) found a 52% decrease in GSH levels in the PFC of schizophrenia patients. Interestingly, a significant deficit in total antioxidant status was inversely associated with some domains of cognitive deficits in schizophrenia patients, such as attention and immediate memory (Zhang *et al.*, 2012). Moreover, plasma SOD activity was negatively correlated with positive symptoms in first-episode schizophrenia patients (Wu *et al.*, 2012). Lower levels of total antioxidant status, catalase and glutathione peroxidation has been described in first episode schizophrenia patients, with GSH levels positively associated with executive function (Martinez-Cengotitabengoa *et al.*, 2012). These findings emphasize the role of oxidative damage in the symptomatology of schizophrenia.

It has also been shown that metabolism of 5-HT, glutamate and DA play important roles in mediating redox balance within biological systems (Smythies, 1999). DA, via monoamine oxidase (MAO) activity (Maker *et al.*, 1981), or oxidized DA through redox cycling (Brunmark and Cadenas, 1988), induces the generation of H_2O_2 and O_2^- , which are known to evoke lipid peroxidation and cell damage, DNA modifications and protein oxidation (Grima *et al.*, 2003 for review). Thus, the efficacy of antipsychotics and their ability to target DA metabolism via blockade of D_1/D_2 receptors may play an important role in suppressing ROS formation and thus present with indirect antioxidant properties that may play a contributory role in the eventual therapeutic efficacy of these drugs (Grima *et al.*, 2003). The release of glutamate on the other hand and subsequent increased calcium entry into cells results in further ROS production (Olney, 1989; Hirose and Chan, 1993). Animal studies, too, have confirmed that schizophrenia involves redox imbalance and oxidative stress (Möller *et al.*, 2011; Radonji *et al.*, 2010; review by Powell *et al.*, 2012). In line with these observations, are recent studies indicating a decrease in parvalbumin-interneurons (PV-IR) expression in the hippocampus, of the ketamine induced model (Harte *et al.*, 2007) and prefrontal cortex, associated with elevations in nicotinamide adenosine dinucleotide phosphate (NADPH) oxidase 2 (Nox2), in the SIR model (Schiavone *et al.*, 2009), which is a major source of ROS and controls glutamate release in the prefrontal cortex (Sorce *et al.*, 2010; reviewed in Schiavone *et al.*, 2012).

The aforementioned studies are therefore adamant that both oxidant and antioxidant systems and redox balance play a pathophysiological role in schizophrenia. This has opened the door to the possible clinical utility of antioxidant drugs (for e.g. N-acetyl cysteine (NAC), discussed in section 8), in the treatment of this disease alone and as an adjunctive treatment (e.g. Adler *et al.*, 1998; Zhang *et al.*, 2001). However, it remains unclear if oxidative stress evident in schizophrenia is due to excess production of ROS or deficient antioxidant mechanisms, or what the source of raised ROS may be.

5.5 Inflammatory mechanisms

A pathophysiological role for immunologic abnormalities in schizophrenia was first hypothesized over 40 years ago (Heath *et al.*, 1967a-c). The association between immunology and schizophrenia, including areas such as neuroplasticity, genetics, and cytokines, has gained an interest (Miller *et al.*, 2011). Cytokines are key signalling molecules of the immune system that are capable of crossing the blood–brain barrier (BBB), and therefore exert effects in both the central nervous system (CNS) and peripheral tissues (Miller *et al.*, 2011). Interleukin (IL)-1, IL-6, interferon (IFN)- γ and tumour necrosis factor (TNF)- α are considered pro-inflammatory as they augment the immune response to infection and inflammation by promoting leukocyte recruitment to inflammatory sites and/or by activating inflammatory cells (Potvin *et al.*, 2008). The primary reservoirs of pro-inflammatory cytokines are the microglia (resident macrophage of the brain) that acts as antigen presenting cells in the CNS (Monji *et al.*, 2012). Microglia can be activated by damage-associated molecules such as ATP (discussed in section 5.7), and contribute directly to the neuronal degeneration via release of pro-inflammatory cytokines and ROS (Monji *et al.*, 2012). In turn ROS play an important role in modulating inflammation (Bitanirwe and Woo, 2011). In this way pro-inflammatory cytokines can inhibit neurogenesis in vivo (Iosif *et al.*, 2006; Kaneko *et al.*, 2006) induce apoptosis (Buntinx *et al.*, 2004; Medina *et al.*, 2002) and adversely affect synaptogenesis, synaptic plasticity and connectivity, and the composition of synaptic membranes (Snico *et al.*, 2005; Stellwagen and Malenka, 2006). On the other hand, anti-inflammatory cytokines such as IL-10 and IL-4 dampen the immune and inflammatory response (Potvin *et al.*, 2008) so that an inflammatory state is generally determined by an imbalance between pro- and anti-inflammatory mediators. IL-6 is capable of evoking both pro- and anti-inflammatory response. Indeed the regenerative or anti-inflammatory activities of IL-6 are mediated by classic signaling, where lipopolysaccharide-induced production of pro-inflammatory cytokines, such as TNF is suppressed by IL-6 (Scheller *et al.*, 2011). Whereas its pro-inflammatory responses is mediated by trans-signaling, by initiating the

synthesis of prostaglandin E₂, responsible for fever and the acute inflammatory response phase (Scheller *et al.*, 2011).

Watanabe *et al.* (2010) has proposed that perturbed cytokine signalling plays a pivotal role in schizophrenia and that genetic and environmental factors directly and/or indirectly impair cytokine signalling, leading to abnormal brain development. Moreover, one of the neurodevelopmental hypotheses suggests that prenatal exposure to infection is associated with an increased risk of offspring developing schizophrenia (reviewed in Bitanhirwe and Woo, 2011). In fact an association between elevated maternal TNF- α or IL-8 serum levels and increased risk for schizophrenia in the offspring have been described (Brown and Derkits, 2010; Deverman and Patterson, 2009; Ellman and Susser, 2009). Other clinical studies have found significantly elevated pro- vs. anti-inflammatory cytokines in patients with schizophrenia as well as their first-degree relatives (Martinez-Gras, 2012), as well as a significant elevation in pro-inflammatory cytokines in first episode psychosis patients with a positive correlation between IL-6 and duration of illness (Miller *et al.*, 2011). IL-6 has been found to be significantly increased in early and late stage schizophrenia, with IL-10 decreased in the late stages (Pedrini, 2012). However, a number of studies have described inconsistent effects on plasma/serum cytokines, namely IL-4 (Kim *et al.*, 2004; Rapaport and Bresee, 2010), IL-6 (reviewed in Drzyzga *et al.*, 2006), IFN- γ (Arolt *et al.*, 2000; Kim *et al.*, 2004; reviewed in Drzyzga *et al.*, 2006), and TNF- α (reviewed in Drzyzga *et al.*, 2006). Although this disparity has been ascribed to differences in duration of illness or antipsychotic treatment, the general consensus is that schizophrenia presents with excessive secretion of pro-inflammatory mediators, and low secretion of anti-inflammatory mediators (Leonard *et al.*, 2012).

Pro- inflammatory cytokines play an essential role in the modulation of various brain functions (Larson and Dunn, 2001; Anisman *et al.*, 2002), markedly impairing affective, emotional and social functions (Dantzer *et al.*, 2008). Furthermore, a positive correlation between the severity of cognitive deficits and IL-1 β , IL-6 and TNF- α levels has been described in schizophrenia (Liu *et al.*, 2010). Importantly, enhanced DA and NA production following enhanced pro-inflammatory activity (Abreu *et al.*, 1994) has been implicated in the emergence of positive symptoms (Kapur, 2003). One of the emerging neuro-immunological mechanisms linking enhanced pro-inflammatory activity with the pathophysiology of schizophrenia involves alterations in tryptophan metabolism (Müller and Schwarcz, 2007).

5.6 Tryptophan metabolism

Tryptophan is catabolized into kynurenine by two haem-dependent enzymes, namely tryptophan-2,3-dioxygenase (TDO) in the liver and indoleamine-2,3-dioxygenase (IDO) in the central nervous system, lungs and placenta (Stone and Darlington, 2002), illustrated in figure 6. Kynurenine in turn, is then metabolised to either kynurenic acid (KYNA) or 3-hydroxykynurine (3-OHK), following then to anthranilic acid, 3-hydroxyanthranilic acid (3-OHAA) and quinolinic acid (QA) (Stone, 2001). This highly regulated pathway accounts for the metabolism of approximately 80% of non-protein bound tryptophan, the essential amino acid needed for the synthesis of 5-HT (figure 6; Allegri *et al.*, 2003). TDO specifically metabolizes tryptophan, while IDO is responsible for the oxidative metabolism of tryptophan, 5-HT and melatonin (Stone and Darlington, 2002). In the brain, tryptophan catabolism occurs in astrocytes and microglia albeit with 60% of cerebral kynurenine contributed from the periphery (Heyes *et al.*, 1997). QA, a recognized NMDA receptor agonist and excitotoxin, along with 3-hydroxy kynurenine (3OHK), a mediator of neuronal apoptosis, and 3-OHAA, a free radical, are all capable of inducing neurodegenerative changes in the brain (Schwarcz, 2004; Stone, 2001; Myint *et al.*, 2007a), KYNA, on the other hand, is an antagonist at the facilitatory glycine site on the NMDA receptor ion channel, thus possessing potential neuroprotective properties (Guillemin *et al.*, 2007). The activation of IDO by pro-inflammatory cytokines (e.g. INF- γ and TNF- α) in the CNS also leads to increased tryptophan degradation into kynurenine and QA, thereby reducing the bioavailability of tryptophan for 5-HT synthesis (figure 6) (reviewed in Myint *et al.*, 2007b). Hence, increased pro-inflammatory and decreased anti-inflammatory actions in the CNS can contribute to central 5-HT deficiency, which plays an important role in the pathogenesis of depression but also the negative symptoms of schizophrenia (Abi-Dargham *et al.*, 1997; Silver, 2004). It is therefore hypothesized that as a consequence of immune activation in schizophrenia, microglia activation induces QA secretion that results in the apoptosis of the neuroprotective astrocytes, thereby exposing the neurones to the neurotoxic effects of 3-OHK, 3-OHAA and QA (Heyes *et al.*, 1997). An over production of QA in turn may also cause the hyper-stimulation of NMDA receptors leading to the release of ROS that depletes energy stores required for mitochondrial function (discussed in section 5.7), causing a further release of glutamate as well as cellular damage or apoptosis (Betzen *et al.*, 2009). The selective loss of astrocytes combined with the apoptosis of neurons could contribute to the decrease in brain volume that typically characterizes chronic schizophrenia (van Erp *et al.*, 2004), discussed in section 5.5.

Together these metabolites contribute significantly to the neuroprotective-neurodegenerative balance in the brain (Myint *et al.*, 2007a, b). Indeed, clinical post mortem studies in patients with schizophrenia have been found to have elevated levels of tryptophan, 3-OHAA, kynurenine and QA in various brain regions (Torrey *et al.*, 1998; Miller *et al.*, 2008). Moreover, unmedicated and medicated individuals with schizophrenia also have increased CSF and plasma levels of tryptophan (Issa *et al.*, 1994; Ravikumar *et al.*, 2000). Although elevated KYNA levels have been described in post-mortem brain tissue of medicated patients with schizophrenia (Schwarcz *et al.*, 2001), Myint and colleagues have described a significant decrease in plasma KYNA concentrations and a decrease in the neuroprotective ratio in medication-naïve and medication-free schizophrenia patients (Myint *et al.*, 2011).

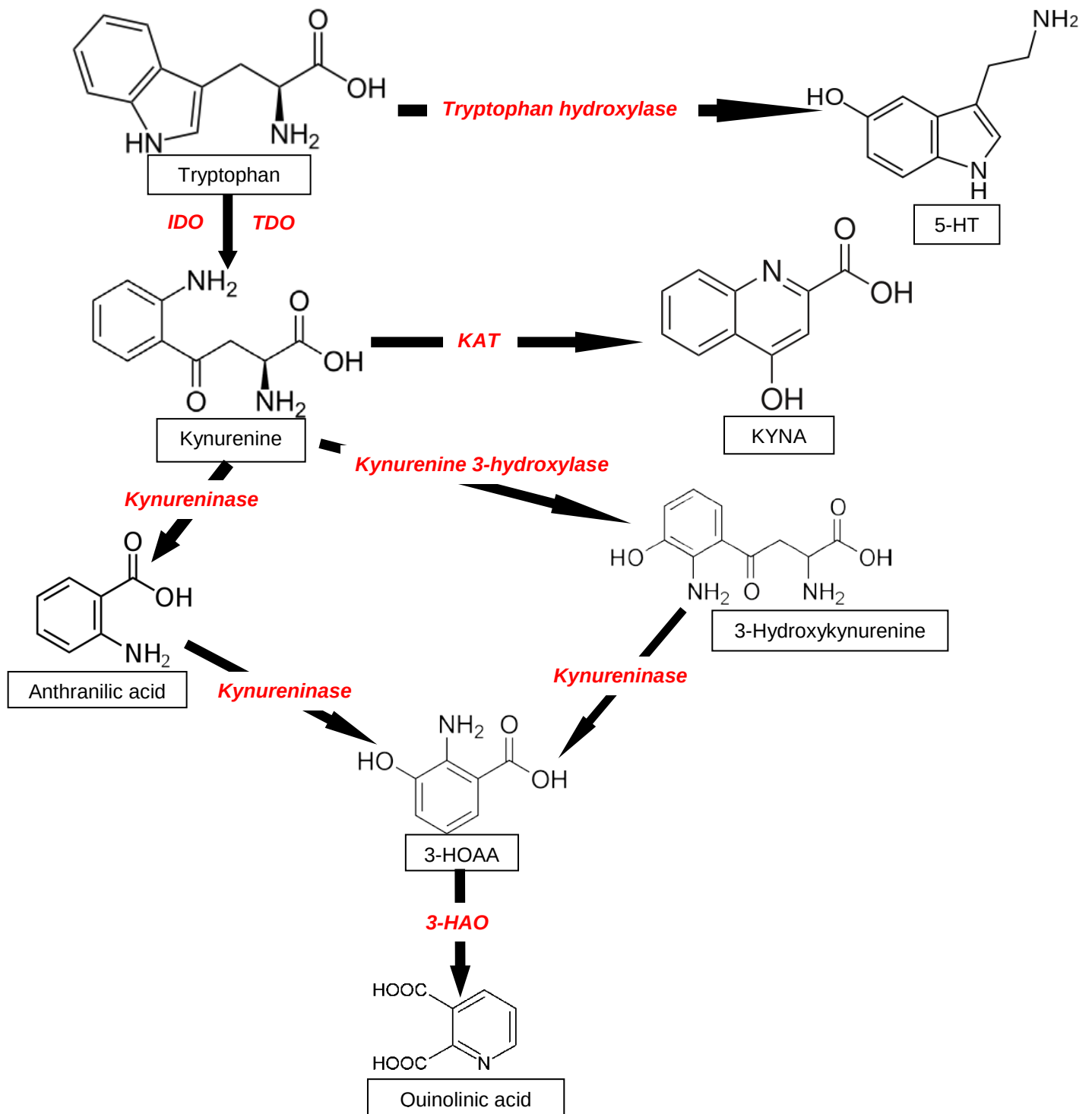


Figure 6: Tryptophan metabolism via the kynurenine pathway. An alternative pathway is tryptophan conversion to 5-hydroxytryptamine (5-HT). KYNA, kynurenic acid; 3-HOAA, 3-hydroxyanthranilic acid; 3-HAO, 3-hydroxyanthranilic acid oxidase; IDO, indoleamine 2,3-dioxygenase; TDO, tryptophan 2,3-dioxygenase; KAT, kynurenine aminotransferase (Adapted from: Stone and Darlington 2002).

The concept of oxidative stress and inflammation in schizophrenia is strongly related to abnormal mitochondrial energy generation. Indeed, altered energy generation is perhaps the oldest biomarker in the disorder (Looney and Childs, 1934), and is discussed next

5.7 Mitochondrial function

The mitochondria is the main source of high energy intermediates required for maintaining energy status in cells, particularly of high-energy consuming cells such as neurons (reviewed in Ben-Shachar and Karry, 2008). Adenosine triphosphate (ATP) is one such high energy source (Jensen *et al.*, 2004). Most cell energy is obtained through oxidative phosphorylation, a process requiring the action of various respiratory enzyme complexes located in the inner mitochondrial membrane, referred to as the mitochondrial respiratory chain (reviewed in Rezin *et al.*, 2009). In most organisms, the mitochondrial respiratory chain is composed of four complexes where the electron transport couples with translocation of protons from the mitochondrial matrix to the inter-membrane space, illustrated in figure 7 (Rezin *et al.*, 2009). The generated proton gradient is used by ATP synthase to catalyze the formation of ATP via the phosphorylation of adenosine diphosphate (ADP) (reviewed in Rezin *et al.*, 2009; Smith *et al.*, 2012) (figure 7). O_2^- molecules are mainly produced at complex four and converted to H_2O_2 by SOD, which in turn is converted to water by catalase, H_2O_2 may also be converted to the free radical, hydroxyl ($OH^\cdot$), responsible for lipid peroxidation reactions (Smith *et al.*, 2012) (figure 7). The mitochondria therefore represent a vulnerable target during oxidative stress under conditions of decreased SOD, catalase, GSH and over production of ROS, as explained in section 5.4.

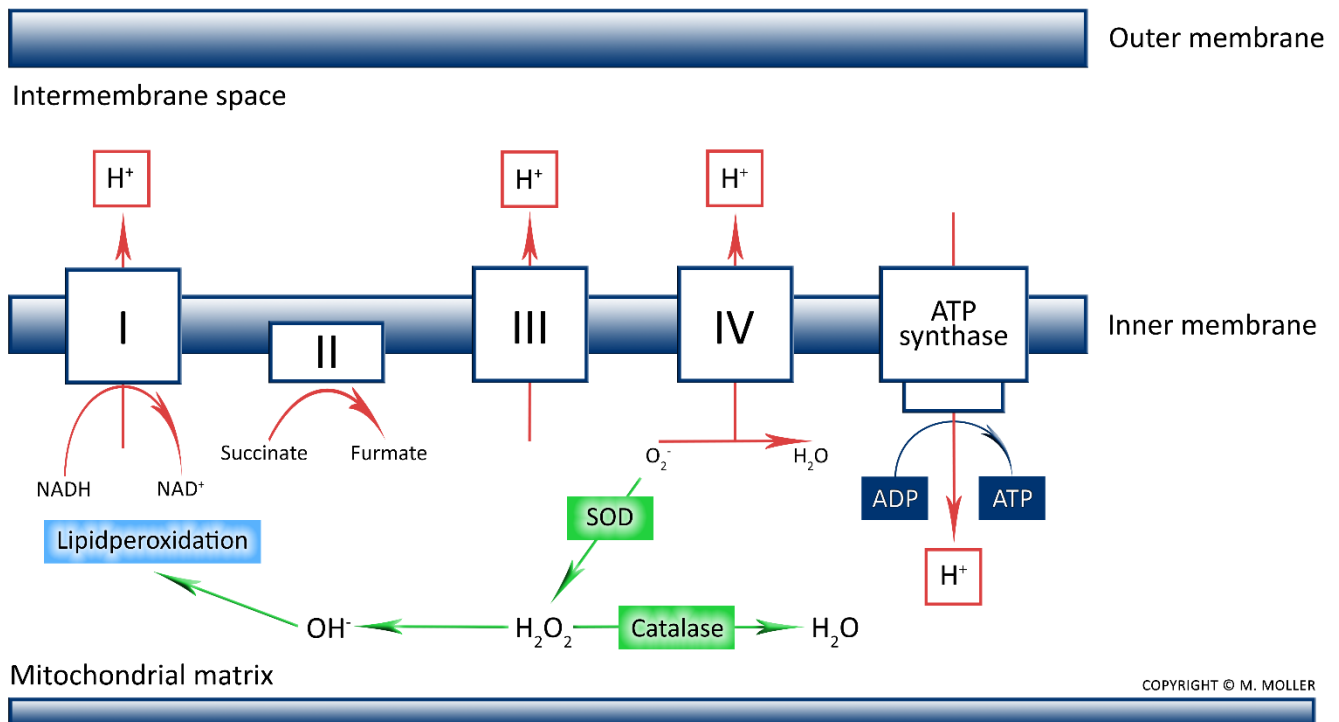


Figure 7: The mitochondrial respiratory chain (complex I – IV), which may lead to increased oxidative stress (Modification from: Rezin *et al.*, 2009; Smith *et al.*, 2012).

Mitochondrial dysfunction and impaired neuronal metabolism can therefore lead to alterations in neuronal function, plasticity and brain circuitry that can be a result of or a cause of abnormal signalling in specific brain circuitries (reviewed in Ben-Shachar and Karry, 2008). Mitochondrial-derived ATP is reduced in several brain structures of relevance in patients with schizophrenia (Volz *et al.*, 2000; Jensen *et al.*, 2006; Fukuzako *et al.*, 1999). Moreover, a correlation between ATP and negative symptoms has been described (Yacubian *et al.*, 2002), while a decrease in complex I subunits has also been observed in the prefrontal cortex and striatum of patients with schizophrenia (Ben-Shachar and Karry 2008), the latter brain regions being key elements in modulating cognitive processes prominent in the disorder (explained in section 5.1). A decrease of ATP in the frontal lobes may therefore indicate an impaired conversion of the energetic substrate into neuronal activity, which is in line with the hypo-frontality observed in schizophrenia (see section 5.1).

The interaction between mitochondrial dysfunction and neuronal transmission may also influence neurotransmitter release, mainly glutamate and DA (Ben-Shachar and Karry 2008). In line with this is that monoamine oxidase, the enzyme responsible for the metabolism of DA is located on the outer membrane of the mitochondria (Ben-Shachar 2002). Previous animal studies have also indicated that DA release in the NAcc is facilitated by endogenous ATP (Krügel *et al.*, 2001), while elevating rat brain DA with L-3,4-dihydroxyphenylalanine (L-DOPA) or d-methamphetamine reduces striatal ATP (Chan *et al.* 1994). These studies suggest that endogenous ATP (and mitochondrial function in general, including redox regulation) reinforces DAergic functions and represents an important target in schizophrenia research.

6. Treatment

Schizophrenia cannot be cured and is a life-long disorder that is progressive over time (Peuskens *et al.*, 2004). Protracted treatment is therefore necessary. Current drug treatment is generally effective against positive symptoms, while negative and cognitive symptoms remain relatively refractory (Kane *et al.*, 1993; Howes *et al.*, 2012). Once these symptoms have been controlled, maintenance treatment can minimize the likelihood of relapse. Despite their apparent effectiveness, up to 40% of patients (depending on how they are identified), fail to show an adequate response to treatment (reviewed in Chakos *et al.*, 2001), while 20% of patients experience a relapse regardless of treatment (Fleischhacker and Hummer, 1997). Moreover, treatment is often complicated by side effects that vary in severity from one patient to the next, and between different drugs (Prior *et al.*, 1999). Side effects may include

extra-pyramidal symptoms, prolactin elevation, sedation and cardio-metabolic effects (Lieberman *et al.*, 2008). Approximately two-thirds of patients on medication for schizophrenia experience persistent Parkinsonism (Harvey *et al.*, 1999), while up to 70% of patients using typical antipsychotics develop acute extrapyramidal side effects (EPS) (Chakos *et al.*, 1994). Previous studies have also indicated that almost all patients experience undesirable side effects during the treatment with antipsychotics (Fakhoury *et al.*, 2001), which unfortunately results in discontinuation or switching of medication (Lieberman *et al.*, CATIE-study 2005; Kahn *et al.*, 2008).

The current treatment regime for schizophrenia mainly comprises the typical antipsychotics that act as antagonists at central D₂ receptors, and atypical or second generation antipsychotics that present with a lower affinity and occupancy for DA receptors but have additional occupancy of serotonergic (5-HT) and other receptors (reviewed in Smieskova *et al.*, 2009). This will be briefly discussed below. However, in the interest of brevity, in-depth discussion of the different agents will be limited to clozapine, the drug that will be the focus in the current study.

6.1 Typical antipsychotics

Chlorpromazine, the first typical antipsychotic, was synthesized in 1950 (Healy, 2003). This drug revolutionized patient treatment, calming down hyper-active patients, ameliorating positive symptoms, and for the first time enabling patients to function moderately well in society. However, beneficial responses were generally accompanied by Parkinson's-like motor disturbances (reviewed in Sawa *et al.*, 2002). This group of drugs includes the phenothiazines (e.g. chlorpromazine), butyrophenones (e.g. haloperidol), thioxanthenes (e.g. thiothixene), dibenzoxazepines (e.g. lozapine), and dihydroindoles (e.g. molidone) (Kane *et al.*, 2009). All examples display high affinity for the D₂ receptor (Marder, 1995). In fact, a strong correlation exists between the therapeutic dose of these drugs and their binding affinity for the D₂ receptor (Kapur *et al.*, 2000). Furthermore, therapeutic doses of these drugs produce high occupancy of the D₂-like receptors in both the limbic areas and the striatum (Xiberas *et al.*, 2001), thus explaining their penchant to induce severe motor side effects at therapeutic doses.

Blockade of 60 – 70% of D₂ receptors is required to reach a threshold of antipsychotic activity, beyond which there is little evidence of enhanced antipsychotic efficacy, except an increase in adverse effects (Kapur *et al.*, 2006). Typical antipsychotics produce a number of problematic side-effects, including extra pyramidal side effects (akathisia, tremors) and

tardive dyskinesia due to blockade of the striatal D₂ receptors. Most of these drugs, with the exception of the butyrophenones, are multi-receptor antagonists, and thus are associated with various cardiovascular, autonomic and other central effects due to non-specific binding to cholinergic, adrenergic and histaminergic receptors (Harvey *et al.*, 1999) (see section 5.3).

6.2 Atypical antipsychotics

The term “atypical” was originally used to describe drugs that predicted antipsychotic effects in animal models but do not produce catalepsy. Clozapine is the archetypal atypical antipsychotic. However, this term was also used to describe drugs that were potentially more effective (particularly against negative and cognitive symptoms) or better tolerated than typical or conventional antipsychotics (reviewed in Geddes *et al.*, 2000).

Cognitive dysfunction in schizophrenia (see section 2.3) remains an unresolved problem in the successful management of the illness, emphasising the need for improved treatment targeted at cognition (Marder and Fenton, 2004). Although second-generation antipsychotics (“atypical”) have an apparent improved negative symptom efficacy over earlier first-generation agents (Lidow, 2000), this has been challenged by the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study. Essentially, this study suggests that first- and second-generation antipsychotic drugs are similar in mechanism of action and efficacy for psychotic symptoms, but also with regards lack of efficacy for avolition and impaired cognition (Keefe *et al.*, 2007). It is only when considering adverse effect profiles that the second generation agents show superiority. However, clozapine was confirmed the most effective drug for refractory individuals (Swartz *et al.*, 2008).

The introduction of clozapine into clinical psychiatry enabled clinicians for the first time to address both positive and negative symptoms, and also to treat refractory patients (Harvey *et al.*, 1999). Its success led to the development of other atypical antipsychotics, including risperidone, olanzapine, quetiapine, ziprasidone, sertindole and zotepine (Miyamoto *et al.*, 2005). However, there is an emerging consensus that these latter incarnations of clozapine are not as effective overall as clozapine and lack its ability to alleviate symptoms in refractory patients (McEvoy *et al.*, 2006; Kumra *et al.*, 2008). Because of an apparent ability to better address negative symptoms and cognitive impairment (Lieberman *et al.*, 2005; Miyamoto *et al.*, 2003), atypical antipsychotics are nonetheless recommended as first-line therapy for acute and maintenance therapy for schizophrenia (Buckley *et al.*, 2001; as recommended by the National Institute for Health and Clinical Excellence (NICE); Edwards and Smith, 2009). Interestingly, a recent study indicated that clozapine in combination with

electroconvulsive therapy significantly improved positive and negative symptoms and all cognitive measures in treatment-refractory schizophrenia patients (Biedermann *et al.*, 2011).

The pharmacological mechanisms underlying the improved response to atypical antipsychotics remain to be identified, although extensive research in recent years has led to a greater understanding of how these antipsychotics work (Miyamoto *et al.*, 2005). The mechanism of action of the atypical antipsychotics is mainly explained by the 5-HT₂-D₂ antagonism theory (Meltzer, 1989), which suggests that a higher 5HT₂:D₂ binding ratio explains the enhanced efficacy and reduced EPS liability of atypical antipsychotics (Duncan *et al.*, 1999; Harvey *et al.*, 1999; see section 2.5.3). However, other mechanisms are also evident (see Harvey *et al.*, 1999 for review). Clozapine specifically will now be discussed.

Clozapine can be classified as a dibenzodiazepine, and is a known multi-receptor antagonist, with affinity for D₁, D₂, D₄, 5HT_{1A}, 5-HT₂, alpha-1, alpha-2, muscarinic, and histamine-H₁ receptors (Nordstrom *et al.*, 1995; Wetterling, 2001; Harvey *et al.*, 1999). Clozapine's improved efficacy over typical agents for the negative symptoms of schizophrenia can be explained by its significant antimuscarinic actions, alpha-2 antagonism, high 5-HT₂/D₂ ratio allowing D₁ receptor disinhibition and /or its mesolimbic-specific action via D₄ antagonism (Sanyal *et al.*, 1997; Harvey *et al.*, 1999). A recent meta-analysis also indicated that clozapine shows an early response profile, similar in pattern but somewhat larger in magnitude than other antipsychotics (Sherwood *et al.*, 2012). This early response may be initiated as soon as occupancy of D₂ receptors by clozapine reaches 50-65% (Kapur and Remington., 1996). Clozapine has a well established reputation for causing little to no EPS due to a high 5-HT₂/D₂ ratio and its low affinity for striatal D₂ receptors (Nyberg *et al.*, 1996; Kapur *et al.*, 2000, reviewed by Stewart, 2002). Importantly, clozapine occupies more than 80% of cortical 5-HT_{2A} receptors at therapeutic doses in humans (Nordstrom *et al.*, 1995). This property leads to disinhibition of cortical-striatal DA neurons via actions on 5HT_{2A} and 5HT_{1a} receptors, as discussed below, and is the basis for its low EPS profile and benefits in negative symptoms and cognition.

Serotonergic neurons from the dorsal raphe nuclei project to the substantia nigra where they synapse with DAergic neurons. Released 5HT acts on somatodendritic 5HT₂ heteroreceptors on these DAergic neurons to inhibit their firing (see figure 8) (Kapur and Remington, 1996). Similarly, serotonergic projections from the dorsal raphe project via the median forebrain bundle to the striatum and cortex, outlined in figure 8, to inhibit neuronal firing by decreasing synthesis and / or release of DA. The actions of 5-HT are thus to impair striatal and frontal cortical DA function, resulting in parkinsonism and cognitive disturbances

and depression, respectively (Harvey *et al.*, 1999). Clozapine counters these effects by increasing DA release through stimulation of presynaptic $5HT_{1a}$ autoreceptors and thereby attenuating 5-HT release, or by blocking post-synaptic $5HT_{2A/C}$ receptors and this disinhibiting DA function. Furthermore, these effects on $5HT_{1a}$ and $5HT_2$ receptors may also constitute an antidepressant-like action, contributing to its unique efficacy against negative symptoms (Harvey *et al.*, 1999 for review), as well as in treatment resistant depression (Ranjan and Meltzer *et al.*, 1996). Clozapine also has a high affinity for $D_{3/4}$ receptors which are preferentially distributed within the LS, so that by blocking these receptors clozapine is able to effectively treat psychosis without severe motor or cognitive side effects (Liégeois *et al.*, 1995).

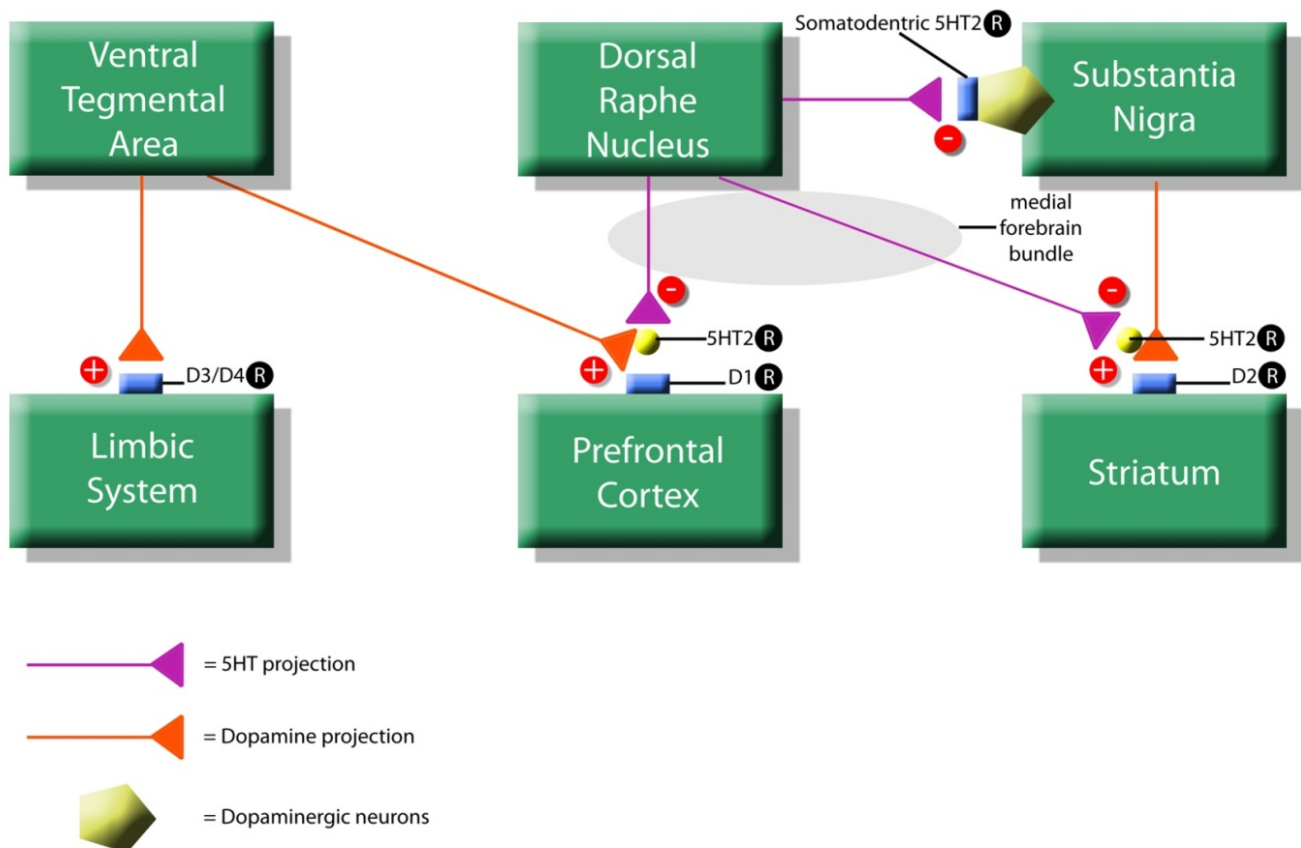


Figure 8: Mechanism of clozapine on serotonergic (5HT) and dopamine (D) receptors (R) in the substantia nigra, limbic system, prefrontal cortex and striatum (Adapted from Harvey *et al.*, 1999 and Möller, 2009).

However, a serious side effect of clozapine is agranulocytosis, which is a significant lowering of the white blood cell count, that could be fatal if not detected early on (Miller, 2000). Other side effects associated with clozapine are cardiomyopathy, constipation, dysphagia, myocarditis, seizures, urinary incontinence, urinary retention and weight gain (Henderson *et al.*, 2000). Clozapine is therefore mainly prescribed for the treatment of persistent psychotic

and negative symptoms, treatment-refractory psychosis and patients with suicidal ideation (Lieberman *et al.*, 2003; Meltzer *et al.*, 2003). A review by Essali *et al.* (2010) recently indicated that in general, clozapine is more effective than typical antipsychotic drugs for treating schizophrenia, and especially for those who do not improve on typical antipsychotic drugs. Moreover, recent evidence suggests that agranulocytosis is more frequent in children, adolescents and the elderly than in young adults or people of middle age (Essali *et al.*, 2010).

6.3 Neurochemical mechanisms in antipsychotic treatment

It has been proposed that activity in the meso-limbo-cortical DA system explains the mechanism of antipsychotic treatment (Casey, 1996; Kapur *et al.*, 2006). Dopamine receptors can be classified into D₁-like (D₁ and D₅) and D₂-like receptors (D₂, D₃, D₄). Moreover, each of these receptor subtypes may have variants that have the potential for explaining heterogeneity in drug responses (Emilien *et al.*, 1999). Moreover, DA neurons project from cell bodies in the VTA and substantia nigra via the meso-limbo-cortical and nigro-striatal DA systems to the nucleus accumbens, amygdala, the neocortex and basal ganglia (figure 9).

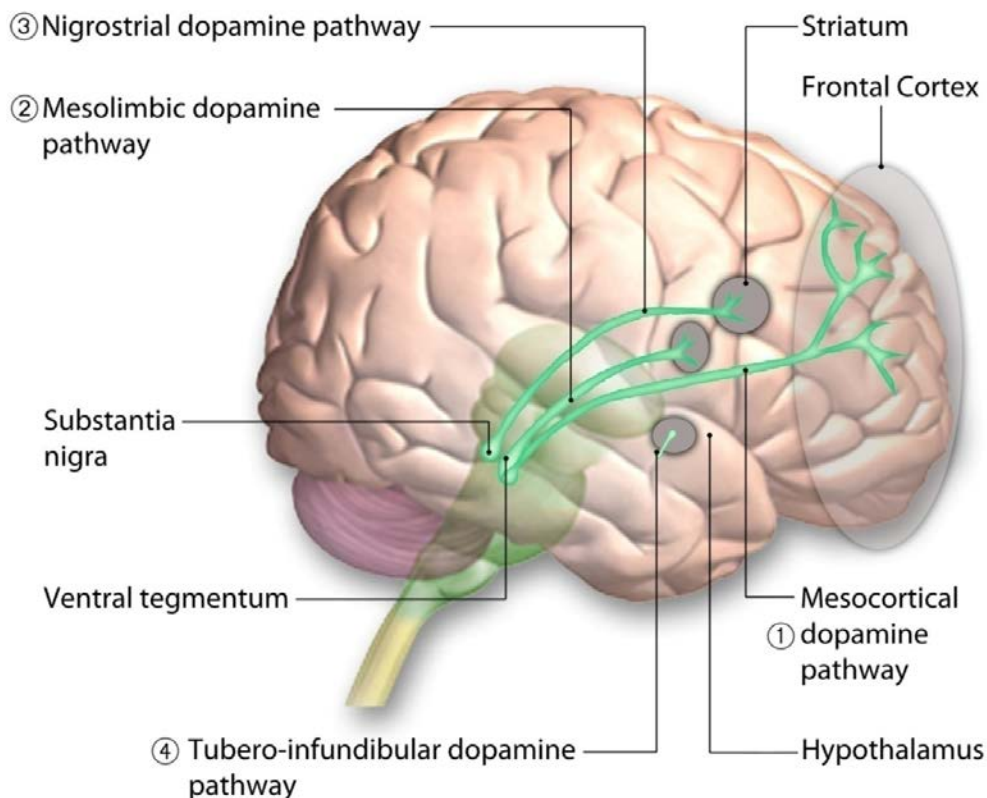


Figure 9: The four principle dopaminergic projections in the brain (Adapted from Leonard, 2003). Pathways: 1, Mesocortical projection. 2, Mesolimbic pathway. 3, Nigrostriatal pathway. 4, Tubero-infundibular pathway.

The meso-cortical pathway (1 in figure 9) innervates the frontal cortical regions, and regulates higher cognitive functions such as working memory, learning and reward (Janhunen and Ahtee, 2007). Hypo-dopaminergic activity of the mesocortical DA pathway is associated with the negative symptoms and cognitive deficits observed in schizophrenia (Knable *et al.*, 1997). Stimulating or sustaining D₁ receptor activity in the frontal cortex will improve deficits in learning, memory and cognition in schizophrenia patients, while blocking this receptor will have the opposite effect (McLean *et al.*, 2009). Thus, for e.g., haloperidol, which blocks both D₁ and D₂ receptors, will cause neuroleptic induced deficit syndrome and possibly worsen negative symptoms (Harvey *et al.*, 1999; Toua *et al.*, 2010).

The mesolimbic pathway (2 in figure 9) innervates the LS and is involved in regulating emotion, reward and motivation (Leonard, 2003). Hyper-dopaminergic activity at D₂ receptors in the NAcc has thus been associated with the positive symptoms of schizophrenia (Carlsson *et al.*, 2004), as well as in mediating the euphoric effects of drugs of abuse, so that by blocking mesolimbic D₂ receptors, an antipsychotic will suppress psychotic manifestations (Keshavan *et al.*, 2008).

Dopaminergic cells in the substantia nigra also project to the caudate putamen via the nigrostriatal system (3 in figure 9), which controls posture and motor behaviour (Janhunen and Ahtee, 2007). D₁ and D₂ receptors are predominant in this brain region, so that disturbances in DA function here will be associated with disorders of motor function, such as Parkinson's disease, dyskinesia, dystonia, stereotypies etc. Blocking striatal D₁ and D₂ receptors will result in EPS and tardive dyskinesia (Janhunen and Ahtee, 2007).

The fourth DA projection is the tuberoinfundibular pathway (4 in figure 9), projecting from the DA cell bodies in the hypothalamus to the median eminence where they release DA into the portal circulation which transports DA to the anterior pituitary gland, and regulates the secretion of growth hormone and prolactin (Dubuc, 2002). Thus, blockade of D₂ receptors in this pathway will result in hyper-prolactinaemia, galactorrhoea and sexual dysfunction (Meaney *et al.*, 2002; Halbreich and Kahn, 2003).

Thus, antipsychotic treatment with preferential activity at meso-limbo-cortical DA sites would result in clinical effectiveness with fewer neurological side effects. However, all antipsychotics have affinity for striatal DA receptors, yet the reason why atypical antipsychotics have fewer EPS is related to their additional effects on 5-HT_{2A} receptors, as discussed earlier (figure 8), which increases DA turnover in the striatum. This same

mechanism explains their beneficial effects on DA functioning in the frontal cortex. In addition, agonism of 5-HT_{1A} receptors also contributes to enhancing prefrontal DA release (figure 8; Ichikawa *et al.*, 2001). Consequently, by acting on serotonergic 5HT_{1A} and 5HT_{2A} receptors, clozapine will augment DA and NA release in the prefrontal cortex and striatum thereby preventing or treating hypo-frontality, improving efficacy for addressing negative symptoms and cognitive dysfunction (Li *et al.*, 1998), and preventing motor disturbances, respectively. This benefit however is not evident in other DA pathways, e.g. the tuberoinfundibular pathway, so that atypical drugs including clozapine remain prone to causing hormonal disturbances and sexual dysfunction (Compton and Miller, 2002).

The prefrontal cortex also contains high densities of 5-HT_{1A} and 5-HT_{2A} receptors located on afferents to and on glutamatergic pyramidal neurons (Martin-Ruiz *et al.*, 2001). Activation of these 5-HT_{2A} receptors promote the release of glutamate (Aghajanian and Mareck, 2000) and thus address diminished cortical glutamate function. Serotonin also inhibits the release of glutamate via the activation of 5-HT_{1A} receptors (Tanaka and North, 1993), so that 5-HT_{2A} antagonism and/or 5-HT_{1A} agonism by clozapine effectively regulates the neurochemical and physiological balance between excitatory and inhibitory inputs onto the prefrontal cortical pyramidal neurons (Millan *et al.*, 2000; Martin-Ruiz *et al.*, 2001), and thus benefits frontal lobe function.

6.4 Additional mechanisms in antipsychotic treatment

Within the context of this study, it is relevant to note certain novel actions of clozapine pertaining to schizophrenia and its association with structural brain abnormalities, oxidative stress, inflammation, abnormal tryptophan metabolism as well as mitochondrial dysfunction (sections 5.1 and 5.4-5.7).

Overall, structural imaging studies suggest that treatment with typical antipsychotics leads to an increased volume of the basal ganglia, while atypical antipsychotics reduce this volume after switching (Smieskova *et al.*, 2009). Moreover, clozapine but not haloperidol treatment re-established normal task-activated regional cerebral blood flow (CBF) patterns in schizophrenia in the anterior cingulate cortex (Lahti *et al.*, 2004). This is consistent with the finding that clozapine (possibly other atypical antipsychotics) might have a greater effect on cognitive impairment in schizophrenia than typical agents, as discussed in section 6.2.

With regards to oxidative stress observed in schizophrenia, increased lipid peroxidation and decreased SOD activity has been described in the brain of rats chronically treated with

haloperidol, but not with risperidone, olanzapine or clozapine (Parikh *et al.*, 2003), while oxidative stress and membrane lipid peroxidation observed in a neurodevelopmental animal model of schizophrenia are reversed by clozapine (Möller *et al.*, 2011). Interestingly, clozapine, olanzapine and risperidone reverse haloperidol induced oxidative stress (Pillai *et al.*, 2007). Clozapine also ameliorates microglia-derived ROS, as well as NO and TNF- α release following lipopolysaccharide-induced neurodegeneration (Hu *et al.*, 2011).

In general, numerous antipsychotics exert modulatory effects on immune function and on peripheral cytokine networks in particular. However, atypical antipsychotics such as clozapine may have more pronounced effects on enhancing anti-inflammatory cytokine signalling compared to the typical antipsychotics (Pollmächer *et al.*, 2000; Drzyzga *et al.*, 2006; Meyer *et al.*, 2011). Thus clozapine, olanzapine, and risperidone, but not haloperidol suppress production of pro-inflammatory cytokines and up-regulates anti-inflammatory cytokines in lipopolysaccharide-treated mice (Sugino *et al.*, 2009), while only clozapine suppresses poly I:C-induced inflammation (reviewed in Monji *et al.* 2012). Moreover, clozapine has distinct yet unpredictable effects on cytokine release in patients with schizophrenia (Lu *et al.*, 2004; Monteleone *et al.*, 1997; Szuster-Ciesielska *et al.*, 2004).

Following discussion on the role of kynurenine metabolism in schizophrenia, clozapine facilitates or inhibits VTA DA neurotransmission dependent on brain KYNA concentration (Schwieler and Erhardt 2003). Interestingly, decreased KYNA levels potentiates the excitatory effects of clozapine on DA neurons in the ventral tegmental area (Schwieler and Erhardt, 2003; Schwieler *et al.*, 2008), ultimately leading to increased DA activity in the frontal cortex and possibly explaining clozapine's efficacy in ameliorating the negative symptoms of schizophrenia. In antipsychotic-free schizophrenia patients, 6-week treatment with antipsychotics was found to increase plasma KYNA levels and decrease 3-hydroxykynurenine supporting a neuroprotective effect of clozapine via the kynurenine pathway (Myint *et al.*, 2011).

Finally, chronic haloperidol and clozapine treatment has been found to increase the levels of γ , α and β -ATP in the brains of rats (Skinner *et al.*, 1995), suggesting that this effect may have relevance to ameliorating mitochondrial abnormalities observed in schizophrenia.

6.5 Other considerations in treatment

A sobering thought is that only 47% of schizophrenia patients are treated with antipsychotics, while 43% of patients receive one additional class of medication, and 10% of

patients receive 2 additional classes of medication in addition to their antipsychotic medication (Cascade *et al.*, 2008). These additional medications comprise mainly antidepressants (28%), mood stabilizers (18%), agents to treat EPS (7%) and sleeping aids (5%) (Cascade *et al.*, 2008), indicating that very often other pharmacological treatments have additional benefits in the successful management of schizophrenia.

The main hurdle in treating schizophrenia is non-adherence to antipsychotic medications, with 50% of patients being non-compliant, which escalates after the onset of the disorder (Fenton *et al.*, 1997). Critical outcomes in the treatment of schizophrenia include improving compliance, to more effectively decrease the severity of psychotic symptoms (desired effect) that is sustained over time with little to no undesired effects, to adequately address negative and cognitive symptoms, and to allow the patient to reintegrate into society (Kasper, 2006). Together these attributes will improve the quality of life of the patient, which is a critical outcome in treatment (see section 8, below). Early and effective intervention in treatment is also critical, as this may improve long-term outcomes. Indeed, first-episode schizophrenia patients respond better to antipsychotic treatment compared to chronic schizophrenia patients, possibly because first episode patients are more sensitive to treatment (Emsley *et al.*, 2008). A randomised, controlled clinical trial also indicated that first-episode schizophrenia patients need to be treated aggressively and that antipsychotic doses can be lower, compared to chronic schizophrenia (Kahn *et al.*, 2008).

On the other hand, with mounting experimental evidence implicating GSH deficiency and oxidative stress (discussed in section 5.4) in the pathophysiology of schizophrenia, novel neuroprotective strategies that aim to limit oxidative stress-mediated cellular damage may also be a valuable treatment strategy to improve treatment response and quality of life (Shungu, 2012). NAC, as a precursor of glutathione and antioxidant, presents as a novel treatment strategy in schizophrenia, as will be discussed below. NAC also forms a central part of this study.

7. N-acetyl cysteine (NAC)

7.1 Chemistry and synthesis

NAC is a white to white-yellow cast powder with a melting point of 109-110°C and the following molecular formula: $C_5H_9NO_3S$ (Sigma-Aldrich, N-acetyl-L-cysteine, 2012). Commercially available NAC for research purposes is prepared from the acetylation of cysteine from human hair (Sigma-Aldrich, N-acetyl-L-cysteine, 2012).

7.2 Physiology and function

NAC is an aminothioliol-containing antioxidant that has been used therapeutically for five decades (reviewed in Cotgreave, 1997). It has been used extensively as a mucolytic agent, in the treatment of acetaminophen toxicity, as a cytoprotective agent during cancer chemotherapy, and in the prevention of contrast-induced nephropathy (reviewed in Cotgreave, 1997; Fishbane, 2008). The mechanism of NAC's antioxidant activity likely stems from its oxygen free-radical scavenging properties and/or its role as a source of cysteine necessary for the biosynthesis of GSH, an important antioxidant system in humans and animals (Dodd *et al.*, 2008; Atkuri *et al.*, 2007). NAC crosses the blood-brain barrier in sufficient quantities to deliver cysteine to brain cells for in situ synthesis of GSH, leading to improved oxidative stress profile (das Neves Duarte *et al.*, 2012). In addition, NAC also supplies cysteine as a front-end substrate for the glutamatergic system, thus modulating the glutamatergic system (Sansone and Sansone, 2011). NAC also possesses anti-inflammatory functions by decreasing TNF- α and IFN- γ levels in rat models of both brain injury and focal cerebral ischemia (Chen *et al.*, 2008; Khan *et al.*, 2004). Another mechanism of action is by reducing apoptotic events in the context of mitochondrial oxidative stress by normalizing factors such as pyruvate and lactate levels (Giulivi *et al.*, 2010).

7.3 Pharmacology

NAC is available as an oral solution as well as intravenous and inhaled preparations, with a half-life of approximately 5.6 hours. It is deacetylated by the liver to cysteine and 30% of the drug is excreted renally (Drug Bank, 2012). Side effects are generally mild; however, there are reports of renal stone formation, drowsiness, stomatitis, clamminess, rhinorrhea and hemoptysis (Drug Bank, 2012).

7.4 Adjunctive treatment in psychiatry

Through NAC's role as a modulator of the glutamatergic system, it has the potential to modulate various critical glutamate-directed events, including modulating the release of DA and other monoamines (see section 5.2) and its subsequent effect on the reward-reinforcement pathway, cognition and mood. Furthermore, due to its regulating actions on the GSH system, NAC will affect the function of a number of redox systems in the body, such as DA metabolism, mitochondrial function and inflammatory processes. As a result NAC may exert a therapeutic affect in a number of psychiatric disorders, especially those

related to oxidative stress (eg. schizophrenia, bipolar disorder, as well as depressive and anxiety disorders) (reviewed in Sansone and Sansone, 2011). Indeed, NAC in combination with antipsychotics (60% being clozapine) was found to increase GSH plasma levels, improve negative symptoms and reduced side effects (akathisia) in schizophrenia patients (Berk *et al.*, 2008a). Bulut *et al.* (2009) also reported a single case of a patient with treatment-resistant schizophrenia who improved significantly following supplementation with 600 mg/day NAC as adjunctive treatment. A study by Lavoie *et al.* (2008) in turn observed significant improvement in mismatch negativity as well as increased GSH plasma levels in schizophrenia patients following adjunctive treatment with NAC. Berk *et al.* (2008b) also noted significant improvements in mood, social and global functioning in 75 depressed bipolar patients receiving NAC as adjunctive treatment. With regards to anxiety disorders, a previous study have reported a favourable response in cases of trichotillomania (hair pulling) following treatment with NAC (Grant *et al.*, 2009). Another study reported notable benefits with NAC as adjunctive treatment in an individual with obsessive-compulsive disorder (OCD) (Lafleur *et al.*, 2006). Thus, NAC offers distinct therapeutic benefits in schizophrenia, as well as other neuropsychiatric illnesses that warrant further research into understanding its mode of action in these disorders and how it may augment the action of current treatments. The fact that NAC is safe, tolerable, affordable and readily available also adds to its interest as adjunctive strategy in psychiatry. NAC will also assist in providing a better understanding of the neuropathophysiology and contributing mechanism in schizophrenia and to develop new and improved treatment regimes, in order to improve the quality of life in schizophrenia patients.

8. Quality of life in schizophrenia

Broadly defined, quality of life can be described as satisfaction in different areas of life, and in objective criteria such as social functioning, activities of daily living and physical health (Lehman, 1988). The quality of life for patients with schizophrenia is associated with overall levels of general psychopathology, increasingly seen as an important indication of daily functioning (Huppert *et al.*, 2001).

People suffering from schizophrenia have a substantially lower quality of life than healthy people (Carlsson *et al.*, 2002), indicating that numerous factors such as compliance with antipsychotic treatment (Coldham *et al.*, 2002), antipsychotic side-effects, increased psychosis, and anxiety may be involved (Yen *et al.*, 2008). In the case of antipsychotic prescriptions, only 58% of prescriptions are filled (Keith and Kane 2003), while 50-75% of

patients on antipsychotic medication become non-compliant after one to two years of treatment (reviewed in Staring *et al.*, 2009). Moreover, previous studies indicated that compliance with antipsychotics has no direct effect on quality of life, but has an important indirect effect through a reduction of psychotic symptoms (Puschner *et al.*, 2009; Staring *et al.*, 2009).

It is therefore crucial for the patient to obtain insight into this debilitating disorder, to realize that self-management of their illness and its treatment through better compliance is a crucial step to reducing debilitating symptoms and to improve psychosocial functioning, ultimately resulting in a better quality of life (Kozuki *et al.*, 2005; Rocca *et al.*, 2009). Depression in these patients adversely contributes to subjective quality of life (Galletly *et al.*, 1997; Huppert *et al.*, 2001). Insight is also related to depression, hopelessness, lower self-esteem (Cooke *et al.*, 2007; Rocca *et al.*, 2009) and lower quality of life (Hasson-Ohayon *et al.*, 2009). A recent study also suggested that increasing the hope of persons with schizophrenia may directly and positively increase both their quality of life and the usefulness of their insight into their illness (Hasson-Ohayon *et al.*, 2009).

Preclinical research on schizophrenia animal models may significantly contribute to identify new biomarkers, improve possible treatment regimes and ultimately contribute to a better quality of life.

9. Animal models

Epidemiological studies have shown increased incidence of schizophrenia in patients subjected to different forms of pre- and perinatal stress (Van den Buuse *et al.*, 2003, discussed in section 5.2). It is not yet fully understood how disruption of early brain development may ultimately lead to malfunction and illness years later. In order to identify the key neurodevelopmental factors in the pathogenesis of schizophrenia, and to highlight potential new targets for novel drug treatment, analogous animal models of schizophrenia are needed (Jones *et al.*, 2011).

Numerous animal models of schizophrenia have been developed, all seeking to replicate one or more of the symptoms of the illness (Dawe *et al.*, 2009). However, no particular model has yet been developed that is able to completely replicate all the symptoms observed in schizophrenia. Numerous behaviours, such as hyper-activity, hyper-locomotion and stereotypic behaviour in animal models are considered to be related to the positive symptoms observed in schizophrenia (Bickel and Javitt, 2009). Although negative symptoms

of schizophrenia are extremely difficult to model in animals, this form of behaviour in man is considered to be related to decreased social behaviour in animals (Meyer and Feldon, 2009). Prepulse inhibition (PPI) and latent inhibition (LI) on the other hand are measures of deficits in information-gating at a pre-attentive sensorimotor reflex level and of attentional habituation and processing respectively (Marcotte *et al.*, 2001; Kapur, 2003). Cognitive functions in animals are often tested by various maze tasks, e.g. the object recognition test (ORT), which is a test of visual recognition memory, and relies on the animal's natural tendency to explore novel environments/objects (Ennaceur and Delacour, 1988). Current animal models of schizophrenia are not intended to serve as an absolute animal equivalent of the human disorder, but rather to assist in evaluating specific causative or mechanistic hypotheses regarding schizophrenia (Marcotte *et al.*, 2001). Before they have utility in pre-clinical research, these models need to be validated according to certain criteria, as described below.

9.1 Validation of animal models

- Face validity: Describes how accurately the model reproduces the clinical core symptoms of the human illness, such as behaviour, as well as the degree of similarity (Marcotte *et al.*, 2001; Meyer and Feldon, 2009) (figure 10). Face validity is also considered to be the most difficult to establish (Marcotte *et al.*, 2001), due to the fact that animals have their own species-specific behaviours, with little resemblance to the behaviours in humans (van der Staay *et al.*, 2006).
- Construct validity: The construct validity of an animal model accesses whether the model is in agreement with current theoretical rationale regarding the human illness it is attempting to model (Marcotte *et al.*, 2001). This form of validity often involves studying the similarity of neurobiological mechanisms in the animal model and the mechanisms known to be involved in the human disorder (Van den Buuse *et al.*, 2003), for e.g. neurochemical and structural defects observed in schizophrenia (figure 10). However, this form of validity has its limitations in what is known about the human disorder, since in many psychiatric disorders the exact neurobiological basis is unknown.
- Predictive validity: Reflects how well the model can predict a given response in the human disorder (Lipska and Weinberger, 2000). In this regard, the ability of the model to identify drugs that have therapeutic significance in humans (or not) is a particularly valuable aspect of validity (Geyer and Markou, 1995). In schizophrenia it is the evaluation of antipsychotic effectiveness and novel therapeutics that take precedence in this criterion (figure 10).

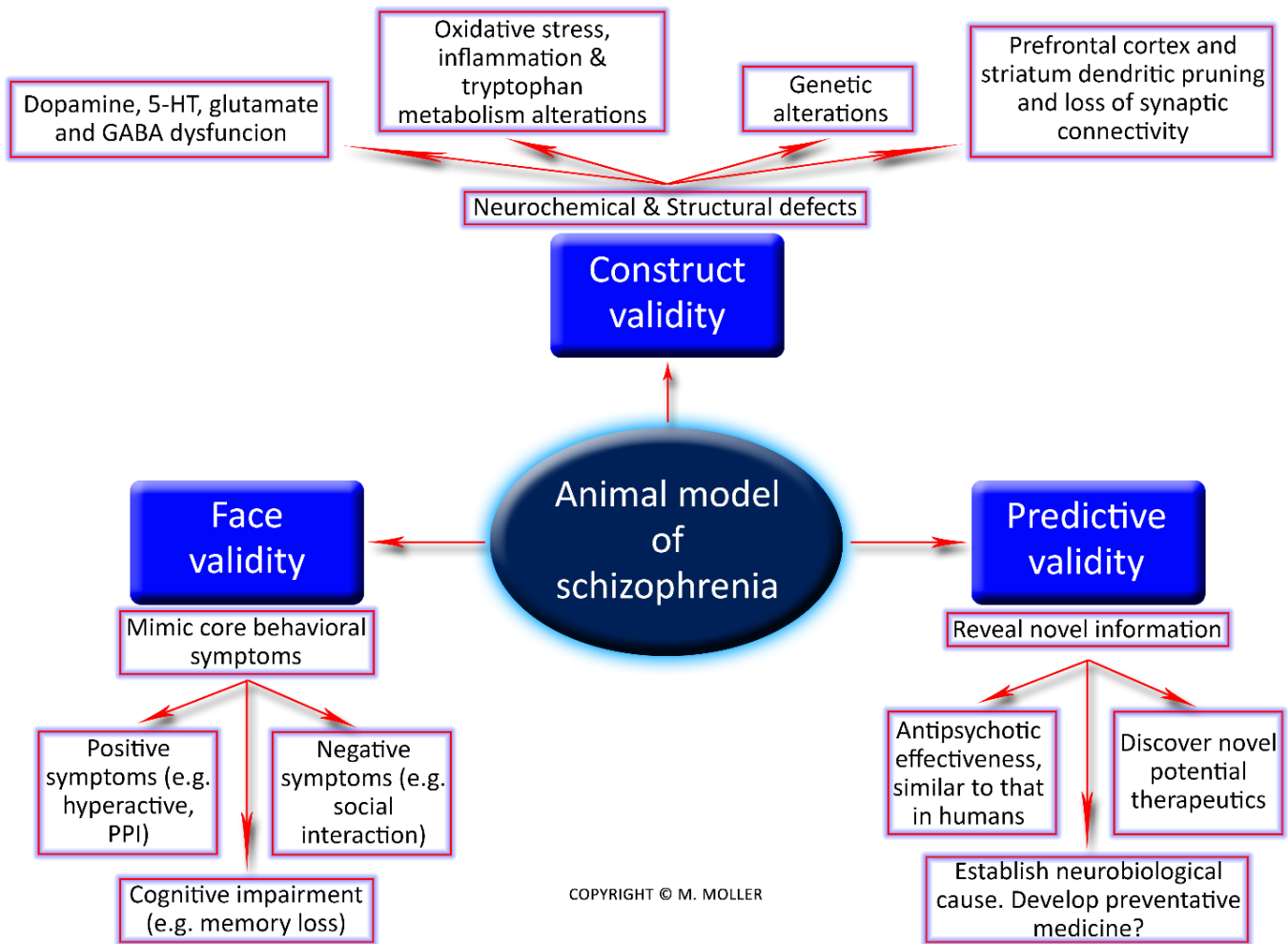


Figure 10: Schematic diagram of the key behavioural, neurochemical and structural changes expected to present and to have translational relevance to the core symptoms of schizophrenia in an animal model of the disorder (Adapted from Jones *et al.*, 2011).

According to the schizophrenia research forum (Taylor *et al.*, 2009), there are approximately 87 animal models that have bearing on schizophrenia, each with a substantial body of published research to back it. However, several of these animal models have considerable overlap in the methodology/principle used, and all fit into four different induction categories: developmental, drug-induced, lesion or genetic manipulation (Jones *et al.*, 2011). However, I will only focus on the social isolation rearing (SIR) model, which is of relevance to my study.

9.2 Social isolation rearing (SIR) as an animal model of schizophrenia

Social isolation rearing (SIR) is regarded as a translational model of schizophrenia, and has been reviewed extensively (Fone and Porkess, 2008; Jones *et al.*, 2011; Powell *et al.*, 2012). Within a colony, rats display social structure and develop hierarchy that plays a critical

impact on their development (Jones *et al.*, 2011). This postnatal developmental model explores the effects of environmental injuries via social deprivation to the developing brain after birth (Ferdman *et al.*, 2007). SIR gained significant interest as a developmental model after it was found to induce pronounced deficits in prepulse inhibition (PPI) in rats (Varty and Geyer, 1998; Heidbreder *et al.*, 2000; Weiss and Feldon, 2001), which is a test of pre-attention sensorimotor gating that also shows impairments in schizophrenia (Braff and Geyer, 1990; Swerdlow *et al.*, 1994). Rearing rats in isolation has since been shown to be a useful paradigm for studying the impact of early life stress on subsequent behavioural changes in adulthood, and also to understand the aetiology of depression and related affective disorders (Heidbreder *et al.*, 2000). Importantly, antipsychotics can reverse PPI disruption in SIR rats (Geyer *et al.*, 1993 for review), providing SIR with important predictive validity for schizophrenia and hence of value for pharmacological studies and for testing antipsychotic drugs (Toua *et al.*, 2010).

Over and above impairments in PPI, SIR also presents with a number of post-pubertal behavioural and neurochemical changes, some of which are similar to those observed in schizophrenia, including locomotor hyper-activity in the open field (Weiss *et al.*, 2000), altered responsiveness to psychostimulants like amphetamine (Hall *et al.*, 1998), heightened anxiety states and aggression (Marsden *et al.*, 2011) and impaired cognitive functions, such as attentional shifting (Schrijver and Würbel, 2001). Recent studies also indicate that SIR rats display impairments in object recognition (McLean *et al.*, 2010), thus reflecting recognition memory deficits that is also observed in schizophrenia (Young *et al.*, 2009). Collectively, these behavioural changes have been termed the “isolation syndrome”, and several of these features resemble some core symptoms of schizophrenia (Geyer *et al.*, 1993; Cilia *et al.*, 2005; Jones *et al.*, 2011). Moreover, we have recently shown in our laboratory that SIR rats demonstrate opposing changes in NMDA and D₁ receptor binding in the frontal cortex of rats with a differential response to haloperidol vs. clozapine (Toua *et al.*, 2010, Addendum B), induces disturbances in markers of oxidative stress in the rat frontal cortex and striatum, as well as deficits in certain core behaviours related to schizophrenia, namely reduced sensorimotor gating and disturbances in social interactive behaviours (Möller *et al.*, 2011, Addendum C). These findings attest to the construct and face validity of the model. Moreover, all these neuro-behavioural alterations can be reversed with antipsychotic treatment, thus providing novel evidence of predictive validity (Toua *et al.*, 2010, Möller *et al.*, 2011, Addendums B and C).

Some of the neurochemical alterations observed following SIR includes reduced cortical DOPAC (3,4-dihydroxyphenylacetic acid)/DA turnover in the frontal cortex (Heidbreder *et al.*,

2000), reflecting lower DA metabolism, while altered frontal cortical D₁ receptor binding (Toua *et al.*, 2010) and increased proportion of striatal D₂ receptors (King *et al.*, 2009) has been noted in SIR rats. SIR rats also show enhanced PFC DA release in response to olanzapine and clozapine, but not haloperidol (Heidbreder *et al.*, 2001). When considering neuroanatomical changes, SIR rats show a significant decrease in the density of dendritic spines in layer 3 pyramidal neurons of the PFC (Silva-Gomez *et al.*, 2003) along with a selective reduction in PFC volume (Schubert *et al.*, 2009) and a decrease PV-IR in the hippocampus and PFC respectively (Harte *et al.*, 2007; Schiavone *et al.*, 2009; section 5.3). PV-IR is a marker specific for GABAergic interneurons in the frontal cortex that, as described in section 5.2, plays a central role in cortical glutamate-GABA-glutamate signalling of relevance for the neurobiology of schizophrenia.

SIR is a pure environmental model that requires no physical intervention to either mother or pup, is relatively simple to execute (Jones *et al.*, 2011), and construct-wise closely correlates with the early life neurodevelopmental hypothesis of schizophrenia (section 5.2). The behavioural and neurochemical changes induced by SIR in rats, as well as their reversal by antipsychotic drugs provide support for a non-lesion and non-pharmacological model with face, predictive and construct validity for schizophrenia. The model therefore has great potential for enhancing our understanding of the developmentally linked emergence of neural and behavioural abnormalities that is characteristic of schizophrenia (Weiss and Feldon, 2001).

10. Conclusion

Schizophrenia is a severe, disabling multifactorial disease involving genetic, environmental and neurodevelopmental factors (Weiss and Feldon, 2001).

Despite the marked improvement of positive, and to some extent, negative symptoms in schizophrenia patients with antipsychotic treatment, lack of efficacy and side-effects hampers compliance and thus favors relapse (Lipkovich *et al.*, 2007). Numerous hypotheses have been developed to define and include the various brain circuits and neurochemical aberrations present in schizophrenia. However, further research is needed in order to improve our understanding of the illness and to develop new and improved treatment, ultimately to improve the quality of life in the patient with schizophrenia. To this end, validated animal models are an urgent need.

One of the most difficult aspects of schizophrenia to model in animals has been the lack of a clear and explicit pathophysiological framework for this illness. Despite the importance and relevance of the neurodevelopmental theory, it has remained difficult to develop specific hypotheses that can be tested in animals (Meyer and Feldon, 2009). Future research should therefore unite the various neurodegenerative– neurodevelopmental and genetic– environmental aspects of the disorder. Multiple factors may contribute to abnormal brain maturation up to puberty, with the ensuing emergence of symptoms and their progression over time. A greater understanding of the various genetic factors involved and the environmental forces that modulate their expression over time may help us to develop more sophisticated animal models. Ultimately, such models may help to expand our knowledge of this poorly understood disorder (Marcotte *et al.*, 2001).

I therefore believe, like many researchers in the field of schizophrenia, that the major challenges facing schizophrenia research is to identify novel biomarkers that will enable us to identify individuals at risk and treat them as early as possible. Neurodevelopmental models, such as SIR, when combined with strong translational measures, may provide a powerful tool to aid this endeavor.

As has been outlined in Chapter 1 section 3 (aims and objectives), the SIR model has not been studied for face validity using three separate behavioural measures performed sequentially that assess core behavioural abnormalities evident in schizophrenia. In this case social interaction, novel object recognition and prepulse inhibition of startle will be studied as an expression of deficits in social behaviour, cognition and sensorimotor gating. In addition, the SIR model has not been studied for construct validity with regards to plasma kynurenine metabolites and cytokines along with changes in regional brain mitochondrial markers and monoamine metabolism. Predictive validity with respect to antipsychotic and antioxidant drug treatment on these bio-behaviours has also not been determined, which will significantly strengthen the validity of the model.

The aim of this study is therefore to investigate a possible relationship between SIR, as a neurodevelopmental model of schizophrenia, and its effects on the above immune-inflammatory, neurochemical and behavioural changes, as well as their response to sub-chronic treatment with the atypical antipsychotic, clozapine and/or the antioxidant, NAC alone and in combination with clozapine. In this regard, I will evaluate if the combination of clozapine and NAC will be equal or superior to that of clozapine alone in reversing these bio-behavioural changes. The study will also provide new information on NAC, especially its ability to effectively reverse bio-behavioural pathology evoked by a neurodevelopmental model of schizophrenia and the dose-dependency of this response, and in so doing provide new insights into its pharmacological effects in psychiatric treatment.